

A message for them . . . or for us?

THE second Voyager spacecraft is due to be launched on its trip to the outer planets on 20 August, followed 12 days later by the first Voyager spacecraft. This apparent paradox is not due to a delay of Voyager I but simply reflects the planned arrival times of the two spacecraft at Jupiter. Voyager I, having taken a faster route, should arrive four months ahead of Voyager II. The nomenclature is therefore sensible. But is the better-known decision, to place a phonograph record on board each spacecraft, also sensible?

As the two spacecraft will fly past the outer planets and then leave the solar system for ever to wander amongst the stars, this provides a unique opportunity to signal our presence to anyone out there by using the Voyager mission as an interstellar message in a bottle. The message consists of a record containing pictorial information about the solar system and its inhabitants, messages in 60 languages and a selection of music. But cold calculation shows that the probability of this message being picked up in the awesome emptiness of space is negligibly small. Measured by its chance of success, this is a futile gesture.

Nevertheless the gesture is worth making; it is an exercise designed more for the benefit of the sender than the supposed recipient and, as Carl Sagan puts it, "the launching of this bottle into the cosmic ocean says something very hopeful about life on this planet". It is an act as apparently pointless but as understandable as leaving a flag on a conquered mountain peak. Objectively the space in the climber's rucksack would be better occupied by an extra pair of socks but, such is the essential optimism and arrogance of the human race, no one objects to such acts.

We can take comfort too from the contents of our message. President Carter states on the record: "We human beings are still divided into nation states, but these states are rapidly becoming a single global civilisation". Presumably he is working on a cosmic time scale—or maybe he knows something we don't. But this process cannot have been accelerated by the omission of reference to the Soviet Union on the record. Although NASA points out that a picture of sprinters has "Valeri Borzov in the lead", this cosmic gesture has missed the opportunity to make a worthwhile worldly one.

Voyager is more than an interstellar envelope, of

course, being part of a programme of planetary exploration. But the programme's future is far from certain, so the inclusion on the Voyager records of the names of the members of the US Congress may ensure a sympathetic response in certain quarters. Similarly, with the costs so great continued public interest is a vital prerequisite for the acceptability of future missions, as Viking showed; the very name Voyager was chosen to fire the public imagination, and NASA takes seriously and performs professionally its tactical duty to educate and explain.

How much more than an interstellar envelope Voyager is, however, is too easily overlooked. The continued emphasis on the search for life has its dangers and has now reached the stage where it has become a betrayal of the broader aims of the scientific community. The tactic did not work with Viking, the common conclusion being that it was an expensive mission that did not provide a clear answer to the one question it supposedly set out to solve. That defines Viking as a failure. Using a similar motivation for Voyager would be a mistake.

NASA does not of course claim that the inclusion of a message is in any sense the motive for the mission, but it is giving the message wide publicity. And this is where the danger lies. The science correspondent who has the tough task of explaining a complicated topic to the public has been handed a gift on a plate: a sellable subject that is easy to write about and, more importantly, liable to get into print. Most science stories get written but not printed. Science writers can use the 'search for life' peg to get a story accepted and then produce a balanced report.

But how many will do this? It happened all too rarely with Viking outside the specialist science press, and so far it has not happened widely with Voyager. The same problem occurs outside the space programme too: how often have we been told, for example, that current research on fusion energy promises inexhaustible supplies of fuel with no radioactive waste? Such myths may help with funding in the short term, but the eventual backlash could be serious.

By the way, we understand that the Voyager spacecraft will send back some sort of data as they pass the outer planets carrying our message into the void. □



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The World Psychiatric Association is about to discuss the misuse of psychiatry. Vera Rich considers the Soviet example

*Orel Prison Hospital, USSR
(Copyright Peter Reddaway)*

WHEN the Sixth World Congress of the World Psychiatric Association (WPA) opens in Honolulu on 28 August, one of the major issues it will discuss is the misuse of psychiatry as a means of repressing political dissent. But with opinions divided over the proper measures needed to combat this abuse, the Congress could face unprecedented controversy.

Some of the seeds of this controversy are to be found in the Soviet Union. Over the past few years, a growing body of information has reached the outside world on the confinement of political dissidents in Soviet mental hospitals. The political abuse of psychiatry is not a specifically Soviet phenomenon—there are reports of 'psychiatric torture' in certain countries of South America, and occasional reports from Eastern Europe of psychiatric measures being taken against would-be emigrants or other protesters. No other country, however, provides such a bulk of material, nor so many well-documented cases.

Public awareness in the West was first drawn to the possibility of political misuse of psychiatry in the Soviet Union in 1965, with the publication in Britain of a book by Valerii Tarsis entitled *Ward 7*. This work was essentially an autobiography disguised as

a novel. Within a few years, several individual cases of such abuse made headline news—notably those of Aleksandr Esenin-Volpin, the mathematician, and Zhores Medvedev, the geneticist. The full extent of the problem only became apparent, however, in March 1971, when the International Committee for the Defence of Human Rights in Paris received over 150 pages of documentation, including what were claimed to be exact copies of official forensic reports on dissidents.

Scrutinised

During the course of the year the documents, which became known as the Bukovskii papers (after their compiler), were scrutinised by Sovietologists and psychiatrists. When the WPA held its Fifth Congress in Mexico in November 1971, strenuous efforts were made to discuss the Bukovskii material. Any firm action, however, was blocked by the Secretary-General of the WPA, Dr Denis Leigh, who averred that nothing in the WPA statutes mentioned that the WPA was responsible for ethical aspects of psychiatry. Nor, he suggested, was there any statute relating to complaints made by one member society against another.

These claims do not seem to agree with the provision in the statutes that

indicates membership of the WPA, both for individuals and societies, may be terminated *inter alia* by suspension. If the WPA cannot deal with such complaints, it is difficult to see on what grounds a member society can be suspended. Dr Leigh contented himself by announcing that the complaints had been "referred to the appropriate quarter, in this case the All-Union Society of Neuropathologists and Psychiatrists"—the very body being complained about. An Ethical Committee was set up to discuss the ethics of psychiatric practice and to draw up an appropriate code of behaviour, to be discussed at the next Congress.

This decision did little to ameliorate things. Indeed, according to former internees, including Bukovskii himself and Viktor Fainberg, conditions for dissidents in mental hospitals, which had been relaxed somewhat immediately prior to the Mexico meeting, became far more stringent once it was clear that the WPA was not prepared as a body to make a stand on this issue. Dr Gary Low-Beer of Horton Hospital, an active member of the Working Group on the Internment of Dissenters in Mental Hospitals, commented that it was not necessary to have a committee to define the ethics of the issue; what was needed was some means of enforcing the accepted ethical standards.

Since the Mexico Congress, the

Bukovskii evidence has been substantiated by a number of other dissident sources, including two Soviet psychiatrists—Semeon Gluzman (now in a labour camp), who exposed the official chicanery that led to a diagnosis of insanity in the case of General Grigorenko, and Marina Voikhanskaya (now resident in London), who refused to administer punitive doses of psychotropic drugs to sane dissidents. According to Dr Leigh himself, even the Soviet delegates at Mexico were prepared to concede that the case reports in the Bukovskii material were authentic. More recently, the Soviet line when releasing a dissident has been that he or she had been insane but was now cured by Soviet medicine. Independent examination of those internees released to the West immediately after discharge from 'hospital' fail to show, however, that any psychosis had been present.

Pattern well known

The general pattern of confinement of political and religious dissidents is now well known: massive punitive doses of drugs, physical restraint, including wrapping the patient in wet canvas which shrinks as it dries, brutal treatment by convict-warders and so on. What still remains in doubt is the stand the WPA should and will take in the matter.

The report of the Ethical Committee will be presented at Honolulu and debated in plenary session. This document will cover a number of issues, including questions of consent to radical forms of treatment, safeguards in the case of involuntary committal, and so forth. So far as is known, the problem of political misuse does not feature specifically, but is contained implicitly in the topic of when treatment is not indicated.

At the General Assembly, however, which represents all 75 member societies, the problem will be specifically raised: the Royal College of Psychiatrists is presenting a motion that the WPA should condemn the Soviet practice of confining dissidents in psychiatric institutions. This motion is not likely to pass unopposed; a number of psychiatrists would prefer to keep 'political issues' out of the Congress, fearing that if the Russians walk out in protest, it would destroy any possibility of diplomatic negotiation. Commenting on this approach, Dr Harold Merskey of the University of Western Ontario observes that the WPA is not an international body like the UN, whose sole purpose is to keep diplomatic channels open; it is a professional body, he says, representing a profession in which ethical standards are all-important in any meaningful

international exchange. He further remarks that "if politics have appeared in the matter, it is the Russians who have introduced them".

A special meeting to discuss individual abuses has been called on the initiative of the American Psychiatric Association; Dr Voikhanskaya is to be one of the floor speakers. The French Committee against Psychiatric Abuse is planning a rally outside the official context of the Congress. The problem underlying all such discussions and protests still remains unsolved: how far is the Soviet psychiatric profession committed to such abuses? And, following from this, what should the stance of individual psychiatrists and psychiatric bodies abroad be?

During the past few years, there has been a certain 'liberalisation' of Soviet psychological and psychiatric theory. This is reflected even in the new (1975) edition of the Large Soviet Encyclopædia. Whereas the previous edition (1955) dismisses psychoanalysis as a "bourgeois pseudo-science", the new version, though still condemning "idealistic" tendencies such as "Freudism", nevertheless acknowledges that the analytic approach has brought into scientific consideration such "important phenomena" as unconscious processes and the effect of the experiences of early childhood.

The historical survey places far less emphasis on Pavlov, who does not even merit a special biographical note, and the former description of psychiatric treatment as the eradication of harmful reflexes and the implantation of healthy ones has been replaced by a less simplistic one. Nor is it any longer stated that psychological "reality" must be understood in the sense of Marxist-Leninist reality. At first glance, the new entries seem almost as hopeful for Soviet psychiatry as the refutation of Lysenkoism was for genetics.

Marxist-Leninist science

Nevertheless, since Marxist-Leninism is considered to be a science in its own right and the basis of all Soviet science the outlook is not promising for those psychiatric 'patients' who hold opposing philosophies. The somewhat bizarre diagnoses such as "schizophrenia with delusions of reformism" may, in practice, be simply a useful tag for dropping a political nuisance into a psychiatric oubliette; but the Soviet psychiatric system provides a background that is particularly amenable to such distortions. The Soviet physicians' oath contains a pledge "to be guided by the principles of communist morality"—principles which specifically put the good of the community above that of the individual.

Certainly, many Soviet psychiatrists

are unaware of the abuses. Dr Voikhanskaya states that she herself knew nothing of them for almost ten years; others, at great personal risk, refuse to participate in them. The problem of political psychiatric abuse in the Soviet Union is not, however, simply that of a relatively small number of psychiatrists putting their devotion to the establishment above their professional commitment; any serious consideration of the problem, or plan to protest, must pay serious attention to the background against which they arise and within which they can acquire some kind of 'socio-political' justification. □

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Bukovskii: sent documents

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Voikhanskaya: son still in USSR

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Gluzman: still held
(This picture and picture opposite courtesy of Peter Reddaway and Sidney Bloch, authors of *Russia's Political Hospitals*, published by Gollancz)

TAIWAN

Losing representation

The council of the International Union of Geodesy and Geophysics (IUGG) met in Durham recently. David Davies reports

In a decision that seems to have been reached amid some concern about the politicisation of international non-governmental scientific organisations, the Council of the IUGG at an extraordinary meeting held in Durham has voted 47-7 to cancel the membership of Taiwan and to bring in the People's Republic of China. The vote is particularly important because it could open the way to much wider Chinese membership of international organisations, though presumably only at the price of much wider expulsion of Taiwan.

Last year the International Union of Geological Science meeting in Australia took a similar course, but the Australian government had not allowed Taiwanese representatives entry visas to state their case. In Durham Professor Y. B. Tsai spoke for Taiwan, but returned with a colleague to Taipei after the ratification of the decision, even though he was scheduled to speak at a subsequent scientific meeting.

The ballot was secret but there is no doubt that the UK and United States voted against. Both these countries stressed that they had no opposition at all to Peking's membership, but only to the preconditions. Indeed, the UK tried without success to get a motion considered which simply admitted Peking whilst taking no action on Taipei. In the event it was clear that many representatives had come with the firmest instructions from their governments or academies to admit Peking on its own terms. A few countries did not even send a scientist to vote for them but despatched an embassy official from London—a practice which the International Council of Scientific Unions would do well to look at as soon as possible.

In hindsight the haste with which the IUGG seems to have brought the matter to a head was probably unnecessary. The next IUGG assembly is planned for Australia in 1979 and it was thought at one time that Australia would refuse to support a meeting at which Taipei but not Peking was represented. But the Australian position has since softened somewhat and it is possible that the Taiwanese would have been admitted provided they did not

claim to be official representatives.

The Taiwan position is difficult now, in spite of assurances that the Bureau of the IUGG will look after the interests of individuals and will ensure that appropriate information gets through. Many Durham delegates urged the Taiwanese to stay, to continue to attend meetings and to remain in the closest touch. But there is a political reason why this is unlikely. The motion that was passed did not simply call for a switch of membership—it pointedly went out of its way to explain the reason: that the "vast majority of countries" now recognise Taiwan to be a province of the People's Republic of China. The Taiwan government also claims that Taiwan is a province of China—but not, of course of the People's Republic. And the Academia Sinica of Taipei is on record as having claimed to speak only for scientists within its own shores. Thus any organisation which acknowledges *de jure* Peking rule over Taipei is unlikely to see Taiwanese citizens at its meetings in the foreseeable future.

The whole sad affair, which gives the impression of having been pushed through for political convenience, will undoubtedly give people ideas about getting rid of other countries because their policies are disagreeable or their political base is weak. □

SWEDEN

Spending a packet

Wendy Barnaby reports from Stockholm on one aspect of Sweden's continuing nuclear debate

LAST March, the Swedish branch of the Friends of the Earth sent a letter to the Prime Minister complaining that a so-called study packet on energy, produced with government funds, was in fact propaganda for nuclear power. The letter provoked some interest. The state auditors dived a little into the financing of the agency distributing the packet, Centrala Driftledningen (CDL), a non profit-making body which promotes many-sided cooperation between the electricity companies, and which is half financed by the State Power Board. The Prime Minister, Thorbjörn Fälldin, replied that he had not known about the distribution of the packet, but that to control the agency's operations would conflict with democratic principles. A question was asked in parliament, but received only a vague answer from the Energy Minister. Then recently—just before the government was to approve the State Power Board's

annual budget—rumours circulated that its normal contribution of about \$865,000 to CDL would be axed. In the event, the money was approved and the agency will be able to continue its normal operations.

The study packet was written by CDL and various organisations responsible for energy supplies. Meant for schools, study circles, trade unions, companies and so on, it consists of seven attractive booklets totalling 152 pages, as well as a 102-page book entitled *Nuclear Energy: Principles and Problems* written by a reactor physicist who works with the Atomic Energy Company. Describing the aims of the package, the distributors said that its facts should be correct and easily distinguishable from evaluations.

In some cases, this holds; often it does not. One of the booklets stresses, for example, that no increase in genetic defects has been found in the offspring of survivors of Hiroshima and Nagasaki. But for a public generally ignorant of the studies showing that the survivors have more than their share of medical complaints, the sus-

picion arises that the authors are trying to minimise the effects of radiation. The same booklet also asserts that the risks of nuclear reactors are small, that the industry is subjected to stricter control than any other, and that the dangers of nuclear-weapon proliferation and terrorism have been exaggerated. In short, the publications, taken together, leave no doubt that the best available energy source is nuclear.

Following the news that CDL would be able to continue, its director, Bengt Sterne, commented: "It was a political question—it's all over now". Of course it was a political question. The President of Friends of the Earth, Lennart Daléus, agrees in principle that CDL should have its money, but says that, as it is the only organisation with enough money to give the public significant amounts of information, it should use government funds more responsibly. Certainly, the problem would be eased if both sides of the nuclear debate could compete on equal terms. It has long been a sore point with anti-nuclear groups that government support is so one-sided. A spokesman at the Department of Industry, which also deals with energy, says that at the moment there is no government fund-

ing of anti-nuclear groups—although there is about \$100,000 in the pipeline for them. A proposal by the Liberal

Party that they should get an annual grant of about \$22,000 seems to have been dropped. Even if it had been adop-

ted, total government spending would still have favoured the pro-nuclear groups by about eleven to one. □

NETHERLANDS

●Mrs Vorrink, Holland's Minister of Public Health and Environmental Protection, plans to eliminate the presence of phosphates in detergents by 1985, according to a report just published by the ministry and already agreed upon in a coordination-committee in which all departments take part. Partial or regional bans operate in Canada and the US; Germany plans to cut back the phosphate content in detergents to half the present level.

The problem is international, and in each country is caused partly by pollution from abroad. In the Netherlands more than half of the phosphates in the surface-waters is imported from other countries, and of the rest a good portion comes from detergents. The initiative to ban phosphates will help, but a more complete solution would require European action. Industry's assistance would also be necessary.

Before Mrs Vorrink reached the end of her ministerial appointment—she is unlikely to return in the new government now in the process of formation—she concerned herself with the warning on spray cans which contain halocarbons. The warning mentions that they can have deleterious environmental effects. The minister's memorandum announces a ban on the use of the gases in spray cans. Industry protested immediately, saying that work for 700 people would be in danger and that the measures would be premature, as no scientific evidence was available that the ozone layer is broken down by halocarbons.

●The former Minister for Science Policy, Mr Trip, will not be part of a new government. What is more, his portfolio is said to be in danger, in spite of advice to the Prime Minister, Mr den Uyl. He has received two reports on improving the communication of information on science and technology to a larger public. One was compiled by two committees, in which scientists, science-information officers and science writers participated.

The product of 18 months' work, the report concluded that a central service for information on science should be created at the Royal Academy of Sciences, which should have a coordinating role. In the Netherlands all universities have their

press- and information-offices; so too do most of the government research institutes. The position has improved in recent years, but gaps remain. Information is restricted



mainly to the institutes' research, and no connection exists with research work elsewhere. The new service has to work as independently as possible and aims at objectivity.

With so many groups working in this area in the Netherlands already, Mr Trip formed another committee to examine whether the government should subsidise existing activities, particularly of foundations which promoted information based on a particular view on society. The committee considered that critical evaluations of the development of science and technology were necessary, and the minister says in his recommendations that more participatory forms of discussion on science are needed, involving various organisations and groups in society. The committee recommends that government money be used for this, without offering criteria for its disbursement. Thus a 'Science for Citizens' programme which is already carried out on a modest scale in the Netherlands may now grow larger.

●Alternative forms of energy receive much attention in this most densely

populated country in the world. After all, nuclear power is not very popular: the construction of three 1,000 MW power plants, and the extension of the ultracentrifuge uranium enrichment plant at Almelo which is part of the tripartite consortium with Britain and Germany, form one of the points of discussion about the formation of a new government. Nuclear matters still dominate research, but alternative energies may obtain a foothold. The minister who has energy in his portfolio, Mr Lubbers of Economic Affairs, is a proponent of nuclear energy on what he calls a modest scale. He has put into service an experimental wind turbine, part of a five-year 15-million-guilder national research programme on wind energy.

The experimental wind turbine, 5 m in diameter, is of the Darreus-type and was built by Fokker-VFW as a spin-off of the rotorblade-production for helicopters. If it is successful a wind turbine 25 m in diameter will be built as well as a more classic wind-turbine of the same size. Mr Lubbers sees only a restricted role for wind energy, however, especially with the environmental and country-planning problems. Experts expect more than 10–20% of future electricity production to come from the wind.

The problem seems even greater with solar energy, but in fact some 50 houses with solar collectors and other energy saving devices have been built in Holland during the last three years. An ecological group, 'De Kleine Aarde' (the small-scale world), began with a recycling house five years ago and the research of a professor at Eindhoven Technical University who lives with his family in a solar house was a stimulus. Philips is doing experiments on 32 houses with collectors; an office building will be constructed with collectors; and this month four houses have been finished in which technical collaborators of the national research organisation will live with their instruments.

The research centre for the building industry foresees the introduction of solar energy devices in houses for space heating and production of warm water from 1980. A national symposium will be held next month on the development of solar energy in the Netherlands.

Casper Schuurin

IN BRIEF

SA nuclear alarm

The question mark surrounding the development of South Africa's capacity to produce nuclear weapons was brought more fully into relief last week following allegations made by the Soviet news agency Tass that South Africa was preparing to carry out weapons tests. The allegations anticipated the rush of diplomatic moves over Southern Africa late last week, and Dr Owen, the UK Foreign Secretary, and Herr Genscher, West Germany's Foreign Minister, each brought the subject up with their South African counterpart, Mr Pik Botha.

Bonn this week denied more reports of collaboration with South Africa on nuclear development. Like allegations earlier this year relating to contacts on nuclear matters between the two countries, these were based on docu-

ments reportedly in the possession of the African National Congress, the exiled black nationalist organisation based in Lusaka. According to the *Observer*, the ANC says that many military visits to Germany have been made under the cover of the South African Council for Scientific and Industrial Research, and are arranged through the scientific counsellor at the Bonn Embassy. ANC officials are also reported as saying that 15 German research institutions, including the Max Planck Institute for Nuclear Physics at Heidelberg, have contributed to South Africa's arms programme.

Committee's report

In its latest report, the UK Select Committee on Science and Technology has examined a particular instance in which British industry did not exploit a

potentially useful innovation. Called 'A case study of machine tool development at the Cranfield Institute of Technology', the report outlines how a high-precision electronic formed-wheel gear-grinding machine, developed at Cranfield Unit for Precision Engineering and Matrix Machine Tools Ltd, failed to gain the financial support needed to develop it commercially.

Blame for this failure is distributed among most of those concerned with the project, though the committee admits that no one acted totally unreasonably. It criticises the Department of Industry's Mechanical Engineering and Machine Tools Requirements Board for not seeking more evidence on the machine's potential market; and the industries involved are criticised for not considering developing the machine as a joint venture.

THE University of California at Berkeley has long prided itself as being a citadel where liberal causes and freedom of thought, action and expression were fostered. Fostered, that is, by a predominantly male faculty. Women did the typing. A less-publicised aspect of campus life at Berkeley is that many women students feel personally insecure. These circumstances lend unusual interest to the appearance of a report, *Women Students at Berkeley: Views and Data on Possible Sex Discrimination in Academic Programs*, that has just been issued by the Office of the Chancellor; 140 pages long, it contains excerpts from academic units' self-evaluation reports and from students' responses to questionnaires.

Chairmen of departments, and other male administrators, made responses that were in many cases masterpieces of low-key diplomacy. The devotion of some respondents to women's liberation was exceeded only by the complete, or nearly complete, absence of women from regular faculty appointments in their departments. Most of us are familiar with the pre-emption of certain scientific fields, such as engineering, by males. It is also evident that physics, chemistry, astronomy and mathematics are predominantly male worlds, in which barriers against women are apparently raised long before college age. However, it surprised me to learn that "Palaeontology is simply a field of endeavour that fewer women than men find attractive, and it is naive to believe that the current disparity in numbers of women to men in the field can be brought to

parity by governmental fiat".

Space permits only a few highlights. The Chairman of the Department of Dramatic Arts notes that "Drama, for 2,000 years, has been predominantly written and acted by

Berkeley women



THOMAS H. JUKES

males . . . Hamlet is a better role than Gertrude, and there are over 30 men and two women in the cast". It might be retorted that Portia, in *The Merchant of Venice*, was much better at making up her mind and getting results than was the moody and vacillating Dane. Also, transvestite males filled women's roles on the stage of Shakespeare's day. Why should not the reverse procedure be tried now?

A laboratory director says, "In all fairness, it must be pointed out that the natural environmental conditions

at the High Altitude Laboratories are physiologically stressful, and for whatever the underlying sociological reasons, the use of the facilities by men has been substantially greater than by women". We hope that his laboratory will provide experimental evidence for the interesting implication that females are more affected by anoxia than are males. I believe that experimental subjects could readily be obtained, for US Governmental Affirmative Action policies have brought many female rangers to the National Parks and Forest Service areas close to the High Altitude Research Laboratory. I have encountered these athletic young females striding along the trails at elevations above 11,000 feet, apparently without signs of stress. Also, women members of the American Alpine Club are more tolerant of high altitudes than they are of suggestions that they are less able than males to adapt to them.

Medicine is a famous battleground of the sexes, and the early women MDs are revered as much as freedom fighters as for their clinical prowess. As every medical student knows, many professors in the clinical sciences are masterful male chauvinists. The report contains several citations of this, and also the following gem: "A neurologist described the Argyll-Robertson pupil 'like a whore who accommodates but does not react'".

Forty-six of the fifty-six female students responding to the questionnaire expressed fear of being raped on the campus at night. Practically none of their professors even mentioned this problem.

news and views

Optimal foraging: theory and experiment

from John Krebs

It may seem a bit optimistic to try and predict in theory how an optimally efficient predator ought to behave, and then expect the predictions of the model to work. Nevertheless a growing body of experiment indicates that the decision rules used by predators are often surprisingly close to those predicted by simple models.

The idea of trying to formulate decision rules for optimally efficient predators was suggested 11 years ago by R. H. MacArthur and E. R. Pianka (*Am. Nat.* **100** 603; 1966) who proposed that predators (using this term in a very broad sense) are designed by natural selection to maximise their net rate of food intake while foraging. They discussed in these terms how an ideal predator might make choices about which food items to eat and where to forage. E. L. Charnov, J. M. Emlen and T. W. Schoener are among several theoreticians who have followed up MacArthur's and Pianka's ideas, and experimental work designed to test the theory has been done, mainly in the laboratory, with birds, arthropods and fish.

Imagine a bird faced with a variety of seeds of different sizes. Ignoring for the moment considerations such as taste and presence or absence of trace nutrients, an optimally efficient bird ought to be capable of ranking the seed types in terms of profitability – the net quantity of food extracted per second of handling time – and when given a straight choice it should prefer the most profitable seeds. There are several studies which have confirmed this basic assumption of optimal diet models for a variety of predators. The predictions of the models relate the extent to which the predator should select only profitable prey and the rate of encounter with the various prey types. In general, predators should be more selective when profitable prey are

encountered frequently than when they are rare. (Note that the term 'encounter rate' covers the effects of camouflage and so on.) This makes intuitive sense. When the predator pauses to handle an unprofitable item, it uses up time which could otherwise be spent in travelling on to the next prey. If profitable prey are sufficiently common it will always be economically advantageous to pass over the less profitable prey. On the other hand in a time of shortage even unprofitable prey may boost the predator's average rate of intake. More precisely, the rule for optimal choice of food items is that the predator should add a new prey type to its diet only if the net food yield per second of handling time (E/h) for this prey type exceeds the predator's current overall net rate of food intake. This prediction can be tested quantitatively by measuring the food values (usually considered in terms of weight or energy), handling times, and encounter rates with various prey types. Particularly noteworthy is the prediction that a predator's decision to accept or reject a new prey type ought to depend only on the value of E/h for the new prey and not on the encounter rate. In other words, there should be (exactly specifiable) conditions in which a predator ignores very common, unprofitable prey.

The importance of this last point can be seen by referring to an experimental test of optimal diet theory in which Werner and Hall (*Ecology* **55**, 1042; 1974) examined the selection of different sizes of *Daphnia* by bluegill sunfish (*Lepomis macrochirus*). Werner and Hall allowed small groups of sunfish to forage in a large tank containing a known mixture of *Daphnia* of varying sizes. They estimated the encounter rate with each size class by making some simple assumptions about the visual field of the fish, and were then able to predict the optimal contents of the sunfishes' diet on the basis of the relative profitability of different sized prey. Large *Daphnia* are more profit-

able than smaller ones because they contain more food but take no longer to handle. Werner and Hall found that when all size classes were available at a low density the fish were totally non-selective, but when the density of all size classes was increased, the fish selectively ignored small *Daphnia*. This switch in behaviour occurred at the encounter rate with large *Daphnia* predicted by the optimal diet model.

More recently O'Brien *et al.* (*Ecology* **57**, 1304; 1976) have pointed out that Werner's and Hall's results could equally well be explained by assuming that the fish obey the rule of always taking the *Daphnia* with the largest apparent size (a small *Daphnia* close by might appear larger than a big one at a distance). O'Brien *et al.* showed that sunfish do in fact take the apparently larger of the two *Daphnia* in a simultaneous choice experiment, and they incorporated these results into a simulation model which predicted a change from non-selective predation at the encounter rate observed by Werner and Hall.

O'Brien *et al.* view their results as demonstrating a proximate rule by which the sunfish achieve the ultimate goal of optimal foraging, but this may be incorrect. The 'take the largest' rule, in agreement with the optimal diet model, predicts that the fish should become more selective as the density of large *Daphnia* is increased, but the two models differ in their prediction of what ought to happen if now the density of small prey is increased enormously. The optimal diet model says that the fish ought to continue to ignore small *Daphnia* even when they are much commoner than large prey, as long as the density of large prey is above the selection threshold. In contrast, the 'take the largest' rule predicts that as the small prey become more abundant, they are more likely to appear larger than big prey to the fish, and hence be selected.

Werner and Hall did not do the test which would enable one to decide

whether sunfish use the 'take the largest' rule, or the optimal diet rule. Two more recent studies have, however, shown that two species of bird will ignore very abundant but unprofitable prey when the encounter rate with profitable sizes is above the predicted level. Goss Custard (*Anim. Behav.* **25**, 10; 1977) showed this with redshank hunting for polychaete worms and Krebs *et al.* (*Anim. Behav.* **15**, 30; 1977) obtained similar results with captive great tits feeding on pieces of mealworm cut into different sizes.

Charnov (*Theor. Pop. Biol.* **9**, 129; 1976) J. M. Emlen (*Ecology; an evolutionary approach* Addison-Wesley, Reading, Massachusetts, 1973), Cook and Hubbard (*J. Anim. Ecol.* **46**, 115; 1977), and Parker and Stuart (*Am. Nat.* **110**, 1055; 1976) are prominent among those who have developed models for optimal allocation of time by a predator to different patches of prey. The models assume that when a predator arrives in a new patch or clump of prey (which might, for example, be a bush laden with berries) its food intake is at first high, but soon declines as the food patch is depleted. The optimal predator should leave a patch when its net rate of food intake within the patch drops to a level equal to the expected intake from the rest of the area. This latter quantity depends on both the average quality of patches and the time and energy cost of travelling between patches. (It is a weakness of both this and the optimal diet models that nothing is specified about how the predator acquires information about its average rate of intake.)

The most straightforward predictions of the model are as follows. First, if

patch quality is held constant, the time spent foraging in each patch should depend on the cost of travelling between patches. The exact relationship between inter-patch travel time and time spent foraging in each patch can be predicted by measuring the curve of food intake as a function of foraging time in a patch. This has been done by Cowie (*Nature* **268**, 137; 1977) using captive great tits foraging for mealworms in sawdust-filled pots. Cowie's results show not only that the time spent in a patch is related to travel time in the way predicted by the model, but that data give a closer fit to the model if one considers net energy intake (subtracting the energy cost of travelling) rather than gross intake. The second testable prediction is that if travel time is held constant and patch quality is varied, the predator should leave each patch within an area at the same threshold rate of food intake, regardless of the initial quality of the patch. This prediction has also been tested and confirmed in laboratory experiments with birds (Krebs *et al.* *Anim. Behav.* **22**, 953; 1974).

In conclusion, although optimal foraging models are in many ways grossly oversimplified, they are good descriptions of the decision rules used by predators in simple laboratory and field conditions. There are many ways in which the models could be elaborated and made more realistic, for example by dealing with the problem of how a predator samples a changing environment in order to assess its average rate of food intake, and how the qualitative differences in food types influence its choice of an optimal diet. □

ratio of secondary cosmic ray muons measured at sea level. Muons reach sea level as the decay products of π mesons and K mesons produced high in the atmosphere from interactions of cosmic ray hadrons with air nuclei. As the primary cosmic rays start off carrying a net positive charge, this is reflected in the excess of positively charged muons at sea level.

Experimentally the cosmic ray sea level muon charge ratio (μ^+/μ^-) is a slow function of sea level muon energy, rising from ~ 1.2 at 10^9 eV to ~ 1.3 at 10^{12} eV. In recent years a number of theoretical attempts have been made to explain this ratio in terms of the known composition of the primary flux and the details of the hadron-hadron interactions. Earlier this year a group from NASA, Badwheer *et al.* (*Phys. Rev. D* **15**, 820; 1977), calculated the sea level differential muon momentum spectrum and charge ratio from 1 GeV/c to 5,000 GeV/c, using available accelerator data. They found excellent agreement between their calculations and existing experimental data and had no need to invoke any change either in the cosmic ray chemical composition or in the nature of the hadron-nucleus interaction at hadron energies above 1.5×10^{12} eV. This result was clearly in contradiction to some earlier conclusions of Adair (*Phys. Rev. Lett.* **33**, 115; 1974).

In view of this discrepancy Adair and his colleagues have now taken the research one stage further by investigating the problem experimentally in the laboratory by observing the charge ratio of muons produced by the interaction of the primary cosmic ray nucleons with the thick atmosphere. The experiment was conducted at Fermilab using the interaction of 400 GeV protons with thick copper targets.

In general, measurements of the charge ratio of muons produced at different energies were made by determining the intensities of positive and negative muons produced by the interaction of 400 GeV protons with copper targets of different mean densities. The charge of the muons was determined by magnetic analysis and the muon energies were measured by determining their range through steel and earth absorbers. For a very dense target the intensity of muons from meson decay is greatly suppressed relative to the atmosphere as a target and sources of muons which do not scale with density cannot be neglected. Such contamination was corrected for by use of targets of differing mean densities. The experiments produced a measured charge ratio for muons of 1.66 ± 0.09 .

These measurements enabled Adair *et al.* to deduce from the cosmic ray energy spectrum that a muon charge

Cosmic ray composition at high energies

from a Correspondent

THE mass composition of primary cosmic ray particles above an energy of $\sim 10^{12}$ eV still remains something of a mystery. At around 10^{11} eV per nucleon very detailed isotopic measurements have now been made, but from 10^{12} eV right up to the highest detected energies of $\sim 10^{20}$ eV per particle controversial debate still rages as to even the simplest question of the proportion of protons in the primary flux. This uncertainty does not reflect any lack of interest or experimental effort attached to the problem as the mass composition has very direct bearing on the origin of cosmic rays, a still very open question. The low primary flux and the Earth's atmosphere combine to make experimental investigation very difficult.

It has long been recognised that at $\sim 10^{11}$ eV $\sim 90\%$ of the primary flux is protons, $\sim 10\%$ α particles and $\sim 1\%$ the rest, largely reflecting the accepted Universal abundance. At $\sim 10^{12}$ eV early satellite experiments by Grigorov *et al.* (*Proc. 12th Int. Conf. on Cosmic Rays* **5**, 1746; 1971) using ionisation calorimeters suggested that the proportion of protons dropped with increasing energy. However the general assumption has usually been that the composition at least up to $\sim 10^{14}$ eV is not too different from that measured at lower energies.

A recent paper by Adair *et al.* (*Phys. Rev. Lett.* **39**, 112; 1977) seems however to support Grigorov's suggestion. This new work is concerned with information deduced from the charge

ratio of 1.50 ± 0.08 should be obtained at sea level for cosmic ray muons of energy $>10^{11}$ eV assuming the accepted primary mass composition. This charge ratio value is significantly higher than the actual measured value at sea level of 1.30. This actual value indicates therefore that $\sim 25\%$ of the total primary nucleon flux must be made up of neutrons. (The primary nucleon flux includes all nucleons whether in multi-nucleon nuclei or not). In other words the flux of heavy nuclei in primary cosmic rays at energies $\geq 10^{12}$ eV per nucleon must be larger than that observed at lower energies.

So this conclusion supports the earlier suggestion from the satellite work of Grigorov *et al.* Such a conclusion if confirmed will be of the utmost importance to cosmic ray physicists. $\square$

Ribosomal switches

from E. G. Richards

THE notion that RNA molecules can exist in several metastable conformational states and that such conformers can be separated by electrophoresis on polyacrylamide gels is by now well known. It all began with tRNA; 5S RNA was the next to exhibit this property and more recently, 16S RNA has been observed to do the same. So far the transformation from one conformer to another has required heat or reagents such as magnesium ions or EDTA. The idea that proteins could do this has remained speculative though proteins have been described that destabilise double helical regions in RNA structures.

These speculations have recently been turned into demonstrations in a paper by Hochkeppel and Craven (*J. molec. Biol.* **113**, 623; 1977). It had previously been shown that 16S RNA isolated from the 30S ribosomal subunits of *Escherichia coli* by treatment with phenol (16S RNA^p) was not the same as the RNA isolated by treatment with acetic acid and urea (16S RNA^a): the two forms can be separated by electrophoresis in agarose/polyacrylamide and 16S RNA^a bound ribosomal proteins that 16S RNA^p did not. In their latest paper they show that if they bind several of the ribosomal proteins to 16S RNA^p, and then remove them again, there results RNA migrating with a mobility close to that of the slower 16S RNA^a rather than that of the faster moving 16S RNA^p. Appropri-

ate controls established that it was not the re-extraction procedure itself that wrought this change. Proteins found to have this effect were S4, S7 and S8, but this of course does not preclude that other untried ones do the same.

These authors went on to demonstrate in a similar way that 16S RNA^p pretreated with S7 (the protein being bound to the RNA and then removed) was able to bind S9 whereas untreated 16S RNA^p was not. 16S RNA^p pretreated with both S4 and S7 was even more efficient at binding S9 but pretreatment with S4 alone did not affect the capacity to bind S9 even though it resulted in a lowered mobility. It would therefore seem that the different ribosomal proteins are able to bring about several different conformational states, with differential protein binding ability but all with reduced mobility.

Further experiments demonstrated that the RNA extracted from the reconstitution intermediate (RI) migrated slowly like 16S RNA^a while that extracted from the heat activated particle (RI*) migrated with the faster mobility. It was similarly shown that inactivation by ion depletion of intact 30S ribosomal subunits results in the extraction of RNA with a lowered mobility as well as a loss of capacity to bind tRNA in the intact particle.

Present understanding suggests that the difference between one RNA conformer and another must reside either in the pattern of base-paired helical regions constituting the secondary structure or in the pattern of folding and interaction deployed in the tertiary structure. It thus seems likely that to change one conformer into another, base pairs or tertiary interactions must first be undone. Several proteins are able to bring this about (including the ribosomal protein S1) so it is not too surprising that the ribosomal proteins, S4, S7 and S8 can do likewise. Nevertheless, the implications for the study of ribosomes are very interesting indeed.

As Hochkeppel and Craven themselves point out, it is now possible to begin to give a rational interpretation in terms of conformational changes in the RNA of the assembly maps presented by Mizushima and Nomura. One can envisage a series of conformational switches each turned on by the binding of a specific protein and each enabling the next protein to bind. If this speculation turns out to be valid, the assembly of a ribosome must be seen as a carefully orchestrated process rather than a series of random associations occurring in no fixed sequence.

The possible importance of conformational switches was discussed a few months ago by Weidner *et al.* (*Nature* **266**, 193; 1977) in a discussion of the

possible conformational states of 5S RNA, another ribosomal component. Recently Bear *et al.* (*Nucleic Acids Res.* **4**, 2511; 1977) have presented some evidence that the binding of proteins L18 and L25 to 5S RNA wreaks a change in the RNA conformation. The evidence is based on changes on the circular dichroism spectra and melting curves of the RNA and RNA-protein complexes and is not wholly conclusive. It does, however, nudge one's prejudices a little further along the direction of supposing that conformational switches may be an important and widely used trick employed in a variety of dynamic molecular processes in living cells. $\square$

New sulphide mineral discovered

from Peter J. Smith

A PREVIOUSLY unknown potassium-rich sulphide mineral has been found by Clarke *et al.* (*Earth planet Sci. Lett.*, **35**, 421; 1977) within a clinopyroxene-ilmenite intergrowth from kimberlite diatremes in South Africa. Until the new sulphide has been more thoroughly investigated, Clarke and his colleagues in Canada and Scotland refrain from giving it a name, referring to it for the time being simply as FSKS (Frank Smith K-sulphide) after the Cape Province diamond mine of which the diatremes form the base. The discovery of a new mineral is not, of course, a completely unheard of event even at this late stage in the development of geology; but what makes FSKS of more than passing interest is that it may indicate an important source of potassium in the upper mantle and throw some light on the vexed question of the transfer of potassium and sulphur from the mantle to the core.

FSKS occurs as ovoid blebs (which also contain pyrrhotite and pentlandite), both as individual grains in cracks in the host clinopyroxene and, more commonly, in close association with the ilmenite lamellae. Because of its high potassium content (8.12–13.01 weight %) it inevitably invites comparison with other potassium-bearing sulphides, namely, the djerfisherite found in several meteorites, the more copper-rich djerfisherite found in the Talnakh copper deposit in Russia, and the rasvumite found in the Russian Khobina massif. But chemical analysis shows FSKS to be quite different from

these minerals. Whereas in djerfisherite the copper contents range from 4–16% and nickel values do not exceed 4%, FSKS contains only 1–3% copper but 11–15% nickel. Moreover, it plots quite distinctly on K–Ni–Fe and K–Cu–Ni ternary diagrams not only from djerfisherite but also from rasvumite.

There seems little doubt, therefore, that FSKS is chemically distinct from other known potassium-rich sulphides. With its primitive cubic unit cell (dimension 10.29 ± 0.03 Å) it is also structurally different from rasvumite, which is orthorhombic (although djerfisherite is cubic in structure). What is less certain, however, is whether or not the significance of FSKS extends beyond the particular circumstances represented by the diatremes within which the mineral is found.

Clarke and his coworkers tentatively propose three possible origins for the clinopyroxene – ilmenite – pyrrhotite – pentlandite–FSKS assemblage, whilst admitting that none is consistent with all the observations. According to the first, the clinopyroxene–ilmenite intergrowth would have grown directly from a silicate melt in the mantle and the polysulphide blebs would have been trapped as immiscible liquid droplets. The unmixing of the blebs into the three distinct phases would then probably have taken place below 600 °C, after the material had been transported to the crust in a kimberlitic fluid. And according to the second, all phases, including the ilmenite, would have been unmixed from an initially homogeneous clinopyroxene by a process of multiple exsolution, although again the separation into the three sulphide phases would have had to have taken place after emplacement in the crust. In both of these models it is implicit that the potassium was present in the initial phases. The existence of FSKS in cracks in the host clinopyroxene, however, suggests the addition of potassium at a late stage, presumably within the diatremes themselves. It is thus possible to envisage a third mechanism in which potassium-bearing sulphides are formed metasomatically at low pressure at the expense of earlier sulphide phases.

If the production of FSKS is really dependent upon diatremes, or on the Frank Smith diatremes in particular, the new mineral clearly has little relevance to the general problem of potassium in the mantle and core. This may indeed be the case, for FSKS has a chemical inhomogeneity great enough to be regarded as uncharacteristic of a phase which has been equilibrated in the mantle for long periods, suggesting that it may be a metastable material associated with

processes occurring during the rise of kimberlitic fluid to the surface. On the other hand, if, as in the first two models proposed by Clarke *et al.*, FSKS is a primary material in the mantle or results from a closed-system unmixing of a monosulphide solid solution, sulphides take on a greater importance as possible storers of potassium in the upper mantle and as possible carriers of potassium to the core during the Earth's differentiation.

This raises deep issues on which it would perhaps be unwise to speculate too much until the properties of FSKS have been examined in greater detail. There has recently been considerable debate and disagreement over the role of sulphur, sulphides and potassium carriers in the Earth's lower mantle and core and any new lead in these matters would be welcome. In the meantime, we have at the very least a new natural sulphide, whatever may be its geochemical significance. □

Pion production in heavy-ion collisions

from P. E. Hodgson

A RECENT measurement of the frequency of pion emission from the collision of heavy ions has given results that disagree with the predictions of the independent particle model of the nucleus and support the pion condensation model of high-density nuclear states.

The frequency of pion production in free nucleon–nucleon collisions is well known, so if nuclei behave just like groups of independent nucleons it is easy to calculate the expected rate of pion production in collisions between two nuclei at high energies. This model might be expected to be valid at energies high enough for the wavelengths of the interacting nucleons to be rather less than the mean separation of the nucleons in the nucleus. If this is so, the nuclei behave for most purposes as if they are groups of independent nucleons, and the expected rate of pion production is given by the total number of possible nucleon–nucleon interactions, making allowance for multiple scattering and shadowing effects. Significant deviations from the expected number of pions would indicate the presence of strong interactions between the nucleons in the interacting nuclei.

The experimental determination of the pion production rate was made by

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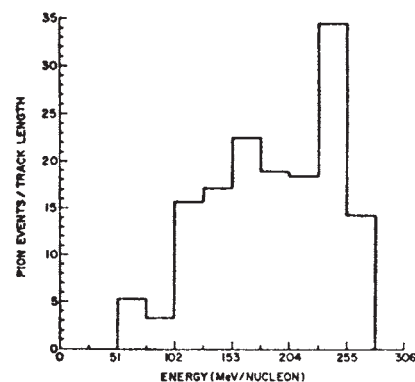


Fig. 1 Pion production as function of neon energy.

McNulty *et al.* (*Phys. Rev. Lett.* **38**, 1519, 1977). They exposed a stack of G5 nuclear emulsion to a beam of 280 MeV per nucleon neon nuclei at the Princeton accelerator. Examination of the developed emulsion under the microscope showed the tracks of these neon nuclei, and 189 tracks were found of nuclei that had collided with a nucleus in the emulsion. Many pion tracks were observed originating in these collision events, and careful subsidiary measurements established that pion tracks could be distinguished from those of protons and alpha particles. The results for the rate of pion production as a function of the energy of the neon nuclei, and also for the pion energy spectrum, are shown in the figures.

A calculation of the rate of pion production expected from the independent particle model has recently been made by Bertsch. He found that the fraction of collisions from which pions emerge should increase from about 1 in 3,000 at the threshold energy of about 54 MeV to about 1 in 27 at energies of 250 MeV per nucleon. This is in complete disagreement with the results shown in the first figure, which show that about 70% of collisions at energies between 100 and 280 MeV per nucleon show pion emission.

Some other calculations of pion production in heavy-ion collisions have been made by Kitazoe *et al.* (*Lett. Nuovo Cim.* **13**, 139; 1975; *Lett. Nuovo Cim.* **14**, 400, 407; 1975) using the

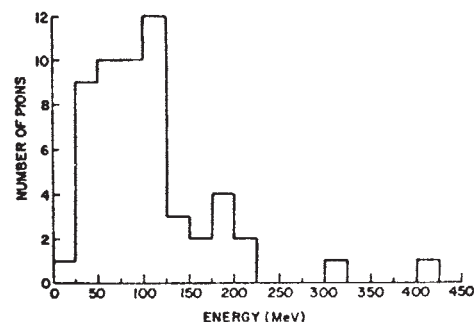


Fig. 2 Pion energy spectrum.

hydrodynamic model to include the collective effects on the formation of high-density states in nuclear matter in high energy heavy-ion collisions. In such collisions it is possible for nuclear matter to be compressed momentarily to very high densities, and this could have a considerable effect on the rate of pion production. The high densities are associated with high temperatures, and these can result in pion condensation in just the same way as a gas is ionised with the liberation of electrons when its temperature and pressure are raised. The probability of pion formation can be calculated from statistical theory assuming thermodynamic equilibrium between the pion and nucleon components. In a later paper they reformulated the theory relativistically and evaluated the rate of pion production quantum mechanically.

In particular they calculated the effect of the condensation of zero-momentum pions, and found that this might be appreciable at incident energies of 120 to 440 MeV per nucleon, reaching a peak at an incident energy of about 220 MeV per nucleon. They estimate that as many pions as 0.4 per nucleon may be produced and allowing for those that do not escape from the nucleus gives a result in agreement with the experimental results.

These calculations are still in an early stage, but it is notable that they give results similar to those found experimentally, while those based on the independent particle model are far too low. This indicates that some new process is taking place in energetic collisions between heavy ions, and this should be a strong stimulus to further experimental and theoretical work. □

The oddity of blue

from J. D. Mollon

A working party was held in Cambridge in 12-14 July, 1977 to discuss the anomalies presented by the 'blue mechanism' of man's colour vision. The meeting was held at the Kenneth Craik Laboratory, an interdisciplinary laboratory for sensory science, recently founded by the Departments of Physiology and Experimental Psychology, and was supported by a grant from the Cambridge Philosophical Society.

THE first stage of man's colour vision is relatively well understood. It is generally held that there are just three classes of retinal cone, each sensitive to a broad band of the spec-

trum; and that all our varied experience of hue depends on the comparison, by later neural mechanisms, of the outputs of these receptor cells, each of which is individually colour blind. However, it is increasingly difficult to neglect a catalogue of signs that the blue-sensitive receptor system differs from the green and red-sensitive systems. By special sensory experiments it is possible to study our visual capacities when stimuli are detected only by blue cones and the 'blue mechanism' defined by these experiments turns out to have many odd properties. Moreover, the blue mechanism is disproportionately vulnerable to retinal disease. The working party was held to attempt to sort out these anomalies.

From the discussions emerged three explanatory principles that may account for most of the anomalies of the blue mechanism. Firstly, there are probably rather few blue-sensitive cones. This might explain the poor quantum efficiency and the poor spatial resolution that characterise the blue mechanism as well as the fact that normal observers when matching very small or brief targets resemble the rare class of congenitally colour-blind observers ('tritanopes') who seem to lack blue cones. H. G. Sperling (University of Texas) presented evidence for the relative rarity of blue cones in the baboon retina: measuring the light-induced reduction of nitroblue tetrazolium chloride in the ellipsoids of cones, he found that the putative blue cones (those that stain strongly when exposed to blue light) constitute 12% of the total population of cones over much of the retina and are arranged in a regular mosaic. D. MacLeod, M. Hayhoe and D. Williams (University of California) have demonstrated sharp local variations in the threshold sensitivity of the blue mechanism within an individual human fovea. They also confirmed the finding of Willmer that the very centre of the fovea essentially lacks the blue mechanism. Correspondingly Sperling's measurements suggest that the proportion of blue cones falls to 3-4% at the centre of the fovea.

A second explanatory hypothesis is that signals from blue cones have access only to the 'chromatically opponent' pathways of the visual system, which are thought to carry information about colour. P. Gouras and E. Zrenner (National Institutes of Health, Bethesda) have detected blue-cone inputs in only one class of retinal ganglion cell, which are characterised by tonic responses to

blue light and off-responses to long wavelengths. P. Whittle (University of Cambridge) tackled the related and vexed, question of whether the blue cones contribute to brightness as well as to hue: presenting to one eye a flash detectable only by blue cones and to the other a white flash on a white field, he found that some subjects were able to equate the two flashes in brightness while others found the task meaningless.

If indeed signals from blue cones are transmitted only by chromatically opponent pathways, it might be expected that the sensitivity of the psychophysically-defined blue mechanism would be controlled not only by photons absorbed directly in the blue cones but also by signals from long-wavelength cones. E. N. Pugh Jr (University of Pennsylvania) and P. G. Polden and J. D. Mollon (University of Cambridge) suggested that a further group of anomalies could be explained if to the second explanatory hypothesis was added a third assumption: an attenuation of the blue-cone signal occurs at the opponent site and this attenuation depends on the difference of the short and long-wavelength inputs, being minimal when the difference is minimal (and when perhaps our sensation is neither blue nor yellow).

With the two assumptions it seemed possible to explain several phenomena discussed in detail at the meeting: the super-additivity of short and long-wavelength fields in reducing the sensitivity of the blue mechanism (Pugh); a newly-reported and paradoxical increase in the sensitivity of the blue mechanism when yellow light is added to a blue adapting field so as to render the composite field brighter but achromatic (Polden and Mollon); the inflexion that is seen in the threshold-versus-intensity function for blue targets on long-wavelength fields (W. S. Stiles); apparent saturation of the blue mechanism (Mollon and Polden); and anomalies of light and dark adaptation (Stiles), such as 'transient tritanopia', the strange loss of sensitivity to blue targets that is found after a long-wavelength adapting field has been turned off and that has now been demonstrated at the ganglion cell level by Gouras and Zrenner and at the level of the b-wave of the electroretinogram by D. van Norren (Institute for Perception, Soesterberg).

Other contributors independently provided results pointing to the second and third hypotheses. Thus R. Boynton (University of California), using pairs of stimuli equated for their effects on both red and green cones and therefore discriminated only by blue cones, has found that such discriminations are impaired by red,

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yellow or green fields that have relatively little effect on the blue cones themselves; long-wavelength fields of different wavelength but of the same luminance produce equal losses of discrimination even though their direct effects on the blue cones are very different. M. Alpern and F. Zwas (University of Michigan) have measured the sensitivity of the blue mechanism to adapting beams passing through different points of the pupil and find that the point of entry for best effect is different for short and long wavelength stimuli, suggesting that more than one class of receptor is involved. They add a fresh anomaly to the

catalogue: unlike the other cones, the blue cones have their maximal directional and spectral sensitivities at the same wavelength.

Inherited and acquired losses of the blue mechanism may not necessarily result from a loss of blue cones. van Norren demonstrated a blue-cone signal in the electroretinogram of congenital tritanopes; and E. King-Smith, F. Zisman and S. K. Bhargava (University of Manchester) have found that the loss of blue sensitivity in disease is greater for flashes on a yellow field than for flashes on a white field, a result that they relate to the second and third assumptions above.

Lampbrush chromosomes brought up to date

from J. O. Bishop

Nearly 100 years after the discovery of lampbrush chromosomes by Walter Flemming in 1882, what is believed to be the first meeting exclusively devoted to them was held at Maria Laach, in the Eifel in West Germany on May 12–15, 1977. The meeting was arranged by Dr Werner Kunz, University of Düsseldorf, and sponsored by the Deutsche Forschungsgemeinschaft.

DURING the first meiotic division of oogenesis segregation of the chromosomes is suspended, in some organisms for months, after the pairing of homologous bivalents and the formation of chiasmata. The oocyte increases greatly in size and it is reasonable to suppose that, whatever the programme for early embryogenesis, some of its most vital components are laid down at this time. The chromosomes become greatly extended and take up the characteristic lampbrush form in which a chromosome axis, condensed chromomeres and extended lateral loops are all clearly distinguishable in the light microscope.

One feature in particular makes lampbrush chromosomes uniquely interesting. The lateral loops are very actively transcribed. RNA complexed with proteins accumulates on the loops. The form of the RNP complexes and their relationship to the loop vary from one loop to another. This, and the fixed positions of the loops on the axis, makes it possible in some cases to identify the same loop in different oocytes. The RNA products of particular loops can sometimes be identified, and the same loop can be studied

at different stages in oogenesis.

Given that a massive increase in the size of the oocyte is necessary, it seems likely that the formation of lampbrush chromosomes is designed to make this possible. The lampbrush form perhaps optimises transcriptional efficiency. On the other hand, since the bivalents are arrested in the process of segregating, perhaps only the parts required for transcription are extended, while the remainder of the chromosome is maintained in a more condensed form. A completely different class of hypotheses is based on the idea that the cell is taking advantage of the growing phase to perform some laborious operation on the chromosomes, such as repair or reconstruction, and that the lampbrush morphology is the result. Of course, these explanations are not mutually exclusive.

It quickly emerged at the meeting that the basis for current thinking about lampbrush chromosomes was laid down between 1950 and 1960, largely due to the work of H. G. Callan in St. Andrews and J. G. Gall

(from the left) O. Hess, H. G. Callan and E. H. Davidson in discussion.



at Yale and Minnesota. It was shown that each loop is based on a single extended DNA duplex. When adjacent chromomeres are separated by physical stress, both remain attached to the ends of the intervening loop, suggesting that there is no disruption of the linear continuity of the chromosomal DNA at the junctions of loops and chromomeres. This widely accepted interpretation requires that the pair of chromomeres lying at the base of a loop must be held together by secondary forces. During the 1950s extensive descriptions of lampbrush chromosome sets were published, and genetic polymorphism of loop morphology was discovered. The latter is a strong indication that lampbrush chromosome structure is at least partly determined by the DNA involved.

Transcription

Study of the structure of lampbrush chromosomes is now intimately bound up with inferences as to patterns of transcription. Electron microscope studies of partially deproteinised preparations (O. L. Miller, University of Virginia, Charlottesville) reveal arrays of RNA fibres emanating from a series of polymerase molecules arranged along the loop. In many cases the lengths of the fibres increase regularly along the loop, and this is very reasonably taken to reflect progressive synthesis. Recent autoradiographic studies on *T. c. carnifex* loops that carry histone-specific RNA (see below) are interpreted to show that transcription is initiated before the histone DNA sequences, proceeds through them and continues beyond, with the histone-specifying sequences being cleaved from the transcripts before transcription ends (Callan). Electron microscope studies of loop-associated RNP particles (J. Sommerville, St Andrews) show them to be composed of surprisingly uniform 20-nm particles. The considerably different appearance of the RNP on different loops is apparently due to differences in the quantity and arrangement of these units.

All the evidence available supports the long-standing idea that the lateral loops are transcribed but the chromomeres and the axis are not. Structures with the appearance of transcription complexes are found only on the loops. Antibody to *Drosophila melanogaster* B-form RNA polymerase binds to the loops, but not to the axis or, apparently, to the chromomeres (E. K. F. Bautz; M. Jamrich, Heidelberg).

Provisionally, a transcription unit can be defined as occupying approximately the region between the shortest and the longest fibres observed in an array of transcription complexes (polymerase + fibril). The arrangement of

For exploring questions of competition and of island biogeography, the aquatic fauna of caves offers the advantages that the communities are relatively simple (usually fewer than 10 species) and that many replicates exist. Culver (*Am. Nat.* **110**, 945; 1976) has used these facts in an elegant study of the aquatic communities in caves in river valleys in West Virginia, Tennessee and Virginia. Those who saw the movie *Deliverance* will wonder whether the NSF underwrote his insurance.

Although flatworms, molluscs, crayfish and salamanders are occasionally found, the macroscopic fauna in these caves is dominated by isopods and amphipods (two of the many orders of crustaceans), which account for more than 90% of the numbers and biomass in most caves. The streams in most Appalachian caves consist of a series of short riffles separated by larger pools. All the isopod and amphipod species prefer riffles to pools, partly because the rate of flow of food particles per unit of cross-sectional area in the stream is higher. The domain within a riffle is a patchy environment, with the habitable patches underneath rocks separated by uninhabitable areas where the creatures are exposed to the current. Since all the species are generalised detritus feeders, the main competition is simply for places to avoid the current.

Culver (*Ecology* **54**, 102; 1973) has previously shown how, under these circumstances, the coefficients for interspecific competition can be estimated for laboratory studies using

Caves as islands

from Robert M. May

artificial streams. The competition coefficients, α , thus obtained are in good agreement with the observed facts about aquatic cave communities: low α -values are associated with species of different sizes (which tend to be under rocks of different sizes); intermediate α -values are associated with species found in different riffles in the same cave; and species never found in the same cave have high α -values. A more detailed analysis of the data available from the laboratory studies shows that communities with three species should be stable and resistant to invasion, which accords with the field observations.

The discussion, based on competition for habitat, explains many of the features of the aquatic cave communities, and suggests most caves could support three species. The average number of species per cave, is, however, more typically two (2.1 species per cave for the Powell River sites, from a total candidate pool of 4 species, and 2.5 species per cave from Greenbriar River sites, from a total pool of 6 species). Culver attributes this to the caves being, in effect, islands. As a consequence, the average number of species per cave is not simply the maximum number consistent with competitive interactions, but rather is some lesser number set by the interplay between extinction and immigration rates.

Such island biogeographical con-

siderations can be worked out in some detail. The upshot of the processes of immigration and local extinction is that any one cave will tend to have fewer species (typically two), and the entire region to have more species (typically four to six), than would be the case if one had the maximum number of stably coexisting species (typically three) in one large cave. This re-echoes a theme first sounded theoretically by Skellam (*Biometrika* **38**, 196; 1951) and Hutchinson (*Am. Nat.* **95**, 137; 1961), with subsequent elaborations by Horn and MacArthur (*Ecology* **53**, 749; 1972) and by Levins and Culver (*Proc. natn. Acad. Sci. U.S.A.* **68**, 1246; 1971). For organisms of this kind, a conservation programme aimed at preserving maximum species diversity would probably be better served by several small refuges rather than one large one (Simberloff & Abele *Science* **191**, 285; 1976). More generally, the optimum pattern for allocation of conservation areas must depend on the life-history details of the target organism.

Culver concludes by emphasising that aquatic cave communities are basically simpler than terrestrial ones. The available evidence indicates, for terrestrial cave communities, that predation rather than competition may play the dominant role, that seasonal changes are more pronounced, and that the available pool of species is greatly affected by local geography. As Culver puts it: 'If you have seen one [type of] cave community, you have not seen them all.'

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these arrays with respect to the loops varies widely, both within and between species. In many cases a single array occupies all or most of a loop, but some contain a single array which does not span the entire loop, and may occupy as little as 30% (K. E. Scheer, Heidelberg). Other loops contain two arrays, sometimes with the same, and sometimes with opposite polarity. Still others contain multiple arrays—5–10% of the loops in *Triturus*, ~50% in the axolotl. When multiple arrays have the same polarity, they may of course represent a single transcription unit with the intervention of one or more processing steps. Where the polarities are opposite this is less likely, and it seems most reasonable to assume for the present that a loop may contain more than one transcription unit.

Organisation

This relates to the relationship between the loops and the axial chromomeres. The proportion of the DNA

present in loops is difficult to measure exactly, but it clearly varies widely from one organism to another. In *Triturus* it is put at between 5 and 10%, in *Pleurodeles* and the axolotl at ~50%. At one time it was speculated that each loop is progressively spooled out from one chromomere (the exit strand) and simultaneously gathered up into the other (the re-entrant strand). This is no longer widely believed, and the loops are thought to be more or less constant. The best evidence for this view comes from *Triturus* where it has been shown that the total nuclear RNA corresponds to a transcript of about 5% of the genome—roughly the proportion of the genome found in loops (D. B. Malcolm, St. Andrews). This is not, of course, a rigorous argument.

Techniques have been developed to pick out the same loop or set of loops in different preparations, and in several cases the functions of particular loops have been positively identified. Of the various methods,

some depend for their specificity on nucleic acid reassociation, others apparently on the recognition of nucleic acids by proteins, and yet others on antigen-antibody interactions.

Histone genes from a number of echinoderms have been cloned in bacterial plasmids and bacteriophage. Nick-translated cloned DNA has been used to search for the histone genes of *T. c. carnifex* (Callan). Of the five biggish loops in the sexually heteromorphic region of chromosome I, three become labelled with these probes in conditions in which the loop-associated RNA is detected. Four other sets of loops on chromosomes VI(2), X and XI are also labelled. Interestingly, differences were observed between the loop-sets of heterozygous bivalents in two cases, and minor differences between different newts are seen. A loop that is not otherwise visible, presumably because it carries little matrix, is visualised

autoradiographically by this technique. What seem to be the same loops can also be labelled (along with others) by using nick-translated middle-repetitive *T. c. carnifex* nuclear DNA, isolated by conventional reassociation methods (H. C. Macgregor, Leicester). The same middle-repetitive fraction of *Triturus* DNA labels six loops near the centromeres of *Plethodon* lampbrush chromosomes perhaps indicating the evolutionary conservation of some families of sequences. A more remarkable conservation is suggested by the labelling of loops in chromosomes II and XI of *Triturus* by human and lung-fish DNA (Gall).

Labelled 5S RNA or DNA of *Notophthalmus viridescens* labels loops near the centromeres of chromosomes I, II, VI and VII (P. J. Pukila, NIMR, London). That of *Triturus c. carnifex* labels a loop on chromosome X. The 5S RNA of the developing oocyte accumulates, together with 4S RNA, in a 40S cytoplasmic RNP particle which predominantly contains only two proteins with molecular weights of 38,000 and 49,000. Antibodies against one of these bind to a loop pair on chromosome X which may be identical to the 5S sequence loop (Sommerville). Antibody against the other binds to several pairs of loops. Antibody raised against a 32,000 dalton protein isolated from the very complex nuclear RNP binds preferentially to 12 pairs of loops. In yet another approach, nick-translated single-stranded DNA binds to two dozen specific loops in conditions that do not favour nucleic acid reassociation, and does so only if RNA has been removed from the preparation (Gall).

Messengers and maturation

Reassociation experiments indicate that the RNA of the oocyte cytoplasm contains only part of the coding potential of the organism. In *Xenopus* the poly(A) RNA of the tadpole contains sequences not found in the oocyte (J. O. Bishop, University of Edinburgh). Similarly, the *Strongylocentrotus* blastula contains mRNA sequences not found in the oocyte (E. H. Davidson, CalTech). On the other hand evidence was presented suggesting that mRNA sequences for both adult and tadpole globin are present at low concentration in *Xenopus* oocytes (M. Rosbash, Brandeis University). It is not really difficult to resolve these results, but we are left without a firm conclusion. On the one side, the reassociation experiments may have failed to detect sequences present in very few copies. On the other, it is possible that sequences with the potential for a very high level of expression also have a relatively high 'leakage' rate when repressed.

Whichever way this question is resolved, it has little direct bearing on the significance of lampbrush loop transcription. Should the oocyte mRNA not contain sequences found elsewhere, those sequences might still be transcribed in the germinal vesicle. A few copies of all mRNAs might reflect a low level of escape synthesis under repression, or synthesis at the derepressed level in an occasional oocyte, rather than synthesis of particular significance.

It is perhaps more profitable to ask to what extent the transcriptional activity of the loops is necessary to account for the RNA present at maturity. Here *Xenopus* and *Strongylocentrotus* are markedly different. A comparison of the poly(A) RNA of stage II (previtellogenic), III and VI (mature) *Xenopus* oocytes by reassociation methods shows them to be qualitatively identical (P. J. Ford, University of Edinburgh). The difference in abundance between the more and less abundant sequences narrows during maturation. Direct measurements indicate that the mRNA synthesised in young oocytes is completely stable. It seems that mRNA is steadily accumulating during maturation, and the synthesis of the more abundant sequences (possibly transcribed from repetitive genes such as histones) is perhaps slowing down. In amphibian oocytes, histone mRNA is largely polyadenylated (M. L. Pardue, Massachusetts Institute of Technology). Calculations based on estimates of the number of active RNA polymerase molecules and the size of the precursor agree that about 3 months would be required to synthesise the number of copies of each sequence found in the mature oocyte (10^6 per oocyte of the less abundant). In *Strongylocentrotus* on the other hand the much smaller complement of RNA can be synthesised in a day, and yet maturation in this organism also takes months (Davidson). A repeated turnover of RNA in the maturing oocyte must therefore occur, which is reflected in the blastula and gastrula: at each stage the bulk of the RNA present is newly transcribed. This applies not only to newly appearing sequences, but also to those common to the previous stage. In *Strongylocentrotus*, therefore, it seems that the accumulation of a store of nascent mRNA for utilisation in early embryogenesis is not a primary function of the period of oogenesis.

This is not to say that nascent mRNA has no part to play. In both amphibians and echinoderms mRNA is mobilised around the time of fertilisation. It is easy to believe that it plays a part at least in the early cleavage stages. About one third of the



A hundred years ago

THE next eclipse of the moon is attracting the notice of physicists and aeronauts in France. It is proposed to make a balloon ascent during the night of August 23-24, in order to ascertain whether, independently of clouds, the eclipsed moon has a bloody tinge.

From *Nature* 16, 16 August, 335; 1877.

oocyte sequences are not found in *Xenopus* tadpoles (Bishop) and in *Strongylocentrotus* two thirds of the sequences found in the oocyte are not found in the pluteus (Hough-Evans).

Spermatocytes

Spermatocytes also show lampbrush chromosomes. In primary spermatocytes of *Chironomus*, lampbrush chromosomes with thousands of loops are clearly visible in the electron microscope but no transcription complexes are seen (H. G. Keyl, Bochum). In spermatocytes of *Drosophila hydei*, however, the Y chromosome forms at least five pairs of morphogenetically distinctive loop structures that closely resemble oocyte lampbrush loops. Apart from the bobbed loci, Y chromosomes in *Drosophila* carry no classical morphological markers. They do, however, carry genetically defined fertility factors, and these have been closely studied in *D. hydei* (O. Hess, University of Düsseldorf). A genetic deficiency of one or another loop is always accompanied by failure of fertility. Some years ago, Hennig (*J. molec. Biol.* 38, 227; 1968) showed that the spermatocytes contain Y chromosome-specific RNA. Also, treatment with actinomycin D causes regression of the loops. In spread preparations transcription complexes are observed (K. H. Glätzer, Düsseldorf). One pair of loops, however, called threads, shows some peculiarities. The pattern of uridine incorporation, the binding sites of RNA polymerase antibodies, and the matrix morphology suggest that the distal parts of the threads are not active in transcription but seem to function as RNP storage organelles. But other explanations are clearly possible.

The relationship of the spermatocyte lampbrush Y chromosome to oocyte lampbrushes is not all clear. However, this system is most promising in its own right, both because of the localised effect of the fertility factors and because a truly cytogenetic approach is clearly possible. □

review article

Taxonomy and the origin and evolution of cultivated plants in the New World

Barbara Pickersgill*

Current taxonomic problems in cultivated plants include delimitation of species, whether speciation has occurred under domestication, and the role of weedy relatives in the evolution of domesticates. Many cultivated plants of the New World seem to have been domesticated not once but several times, and agriculture itself may well have originated independently in four different parts of the Americas.

STUDIES of the origin and evolution of cultivated plants aim to answer four basic questions: from what did our modern crop plants originate; where were they domesticated; when did this happen; and how have these crops changed and spread since they were first taken into cultivation? In 1886, Alphonse de Candolle¹ outlined the pertinent sources of data in order of usefulness: first, archaeology; second, botany; third, history; fourth, philology. Today all these types of evidence are still used, though 'botany' in de Candolle's sense has become the province of taxonomy in particular, and involves more than gross morphology and geographical distribution. Cytotaxonomic studies are now routine, while chemotaxonomy may furnish additional information on relationships. New micromorphological characters have become available with the development of the scanning electron microscope and, together with improved archaeological techniques for the recovery of plant material, are greatly assisting identification and analysis of archaeobotanical specimens. Numerical taxonomic techniques enable taxonomists to analyse variability and assess resemblances using many variables, from many different fields, simultaneously.

Species concepts in cultivated plants

When investigating the origin(s) of a particular crop, the first essential is to know how many species are included in that crop. Although Darwin² and de Candolle¹ called attention to the striking and discontinuous intraspecific variation characteristic of cultivated plants, taxonomists of the period often elevated these variants to the rank of species. With the rediscovery of Mendelian genetics, many of these 'species' were correctly evaluated as simply lines differing in one or a few major genes. The development of genecology focussed attention on the process of speciation, particularly the role of reproductive isolation in the differentiation of species. This likewise led to the recognition of fewer, but more variable, species. The taxonomist's task is to distinguish between discontinuities which may seem very striking but which nevertheless represent simply major gene variants within a cultivated species, and discontinuities which may appear trivial but which delimit boundaries between species.

The effect of these changing species concepts is well illustrated by the chili peppers (*Capsicum*). In the nineteenth century, over 60 species were described, differing mostly in colour, size, shape and

pungency of the fruit. In 1923, Bailey³ reduced these to a single species. Now, five different species of domesticated chili pepper are recognised⁴, mainly by floral characters, since the more strikingly discontinuous fruit characters show parallel variation within the domesticated species.

Speciation and domestication

Improved taxonomic understanding of other crops has shown that not only *Capsicum*, but also cotton, tobacco, beans, squash and amaranth, among others, comprise two or more cultivated species, grown for the same product and used in the same way, but usually with their own characteristic geographical distributions⁵. This poses the problem of whether speciation preceded domestication (which implies that the individual cultivated species were domesticated independently, from wild ancestors which were already specifically distinct), or whether speciation followed domestication (in which case each crop may have been domesticated once only). This question has to be answered separately for each crop, and the answers may well be different.

In cotton, truly wild plants (not escapes) are known in both Mesoamerican *Gossypium hirsutum* and South American *G. barbadense*. The earliest archaeological cotton from South America belongs to *G. barbadense* and seems to represent a very early stage in domestication⁶. The earliest Mexican archaeological cotton is, however, 'almost certainly' *G. hirsutum* and is fully domesticated, although it is older than the earliest Peruvian specimens⁷. These data strongly suggest independent domestication of the two cultivated species of New World cotton from specifically distinct wild ancestors.

Among South American cultivated diploid potatoes, on the other hand, four species have been recognised⁸. *Solanum stenotomum* is the basic, and most variable, species. *Solanum gonicalyx* is a northern derivative which consists of a few relatively invariable clones. *Solanum ajanhuiri* probably results from hybridisation between cultivated *S. stenotomum* and the wild frost-resistant species, *S. megistacrolobum*⁹. *Solanum phureja*, adapted to the wet eastern slopes of the Andes and distinguished by early maturity and non-dormant tubers, was perhaps derived by assortative mating within the original variable cultivated complex¹⁰. Here, domestication has been followed by increasing differentiation, to the verge of speciation, within the cultivated populations.

Chili peppers present a more complex situation. Of the five domesticated species, *Capsicum baccatum* and *C. pubescens* are morphologically and cytogenetically so distinct that they must each have been domesticated independently from distinct wild species.

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Fig. 1 *Helianthemum*. From *Hortus Eystettensis*, vol II, Besler, 1613.



Fig. 2 *Solanum*. From *Hortus Eystettensis*, vol II, Besler, 1613.

Numerical taxonomic studies¹¹ confirm the probable independent domestication of *C. baccatum* but suggest that domesticated *C. annuum*, *C. chinense* and *C. frutescens* all arose from different parts of the same incompletely differentiated complex of wild forms. In chili peppers, therefore, speciation in some instances preceded, and in other instances was accentuated by, domestication.

The role of weedy relatives

Often the closest wild relatives of a cultivated plant prove to be weedy forms in the same, or a closely related, species. As de Candolle¹ noted, distinguishing genuinely wild ancestors from feral descendants of a crop is often very difficult. Again, the problem must be evaluated independently for each crop and the decisive evidence may come from very different sources.

In cotton¹², primitively wild forms have small bolls, often with sharp recurved beaks, and greyish-white or dingy brown lint sparsely covering the small hard seeds, which are dispersed individually. The beak has been reduced in size in hand-harvested domesticated cottons and has not been regained in feral cottons. Feral cottons also retain the clear white or uniformly brown lint of their cultivated ancestors and their lint is usually so abundant that all seeds in one locule of the boll are dispersed as a unit. Critical analysis of morphological features may thus permit primitively wild cottons to be distinguished from escaped feral forms.

In *Cucurbita*¹³, there has been argument over whether weedy *C. andreana* is the wild ancestor or an escaped derivative of the South American cultivated *C. maxima*. With the exception of *C. ecuadorensis*, whose status also seems doubtful, no other wild species of *Cucurbita* is known from South America. *Cucurbita* flowers are pollinated by various insects, including squash bees (*Peponapis*) which are wholly dependent, as larvae and adults, on *Cucurbita* flowers for food. Three species, representing three different subgenera of *Peponapis*, are confined to South America. It seems unlikely that South American *Peponapis* could have diverged as much as this if *Cucurbita* had been introduced by man

(hence comparatively recently) from Middle to South America. Here, then, taxonomic study of the pollinator suggests an ancient presence of more than one species of *Cucurbita* in South America, presumably including *C. andreana*, hitherto considered only doubtfully wild.

Many wild chili peppers also occur in weedy contexts, raising doubts about their relationships to the domesticates. Virtually all accessions of domesticated *Capsicum annuum* belong to the same, derived, chromosome race, which in wild *C. annuum* occurs only in Mexico¹⁴. Other wild forms of *C. annuum* carry the basic karyotype shared by most species in the genus. Cytological data thus strongly suggest that weedy forms of *C. annuum* are primitively wild and that only the Mexican weedy forms are involved in the origin of domesticated *C. annuum*.

Electrophoretic studies on isozyme variability may also help in unravelling relationships between cultivated and weedy races. The New World cultivated chenopods include one tetraploid, *Chenopodium nuttalliae*, in Mexico and one tetraploid, *C. quinoa*, in the Andes. The two are very similar and there has been some doubt as to whether they really represent distinct species. Weedy tetraploids also occur in the Andes (*C. hircinum*) and from Mexico north into North America (*C. berlandieri*). All the tetraploids carry two unlinked genes for synthesis of the enzyme leucine aminopeptidase (LAP) (ref. 15). Virtually all Andean accessions of cultivated *C. quinoa* and weedy *C. hircinum* are homozygous for *Lap-A2* and *Lap-B2*. Mexican cultivated *C. nuttalliae* and weedy *C. berlandieri* are also homozygous for *Lap-A2* but at the *Lap-B* locus they carry *Lap-B1* not *Lap-B2*. In North America, outside the range of *C. nuttalliae*, *C. berlandieri* is polymorphic for *Lap-A* and monomorphic for *Lap-B2*. These studies support the view that *C. nuttalliae* and *C. quinoa* are distinct species; fit with earlier data on genetic control of fruit colour which imply that pale-fruited domesticated forms of *C. nuttalliae* and *C. quinoa* originated independently¹⁶; and suggest that weedy *C. berlandieri*, at least, is more likely to be ancestral to its related cultigen than derived from it.



Fig. 3 Bottle gourd. From the *Herball* 2nd edn Gerarde, 1636.



Fig. 4 *Capsicum*. From *Theatrum Botanicum*, Parkinson, 1640.

Origins of polyploid crops

Many New World cultivated plants are polyploids and here the direction of evolution, from diploid to polyploid, is unequivocal. The initial steps in determining the ancestry of a polyploid are now largely routine, but the results of these investigations are interpreted more cautiously than was the case a decade or so ago. The potato has many attributes of an autotetraploid, with four homologous sets of chromosomes, but forms mainly bivalents at meiosis¹⁷, that is, shows pairing behaviour usually associated with allopolyploidy. Cotton is a classic allotetraploid, but in AD polyhaploids derived from the AADD cultivated cottons there is less pairing between A and D chromosomes than occurs in newly synthesised AD hybrids¹⁸. This suggests a genetic restriction of pairing between A and D chromosomes which exaggerates the apparent difference between the two genomes. Chromosome pairing alone may thus be an unreliable guide to the nature and origin of a polyploid.

Comparative morphology may also be misleading. The most probable ancestor of the cultivated tetraploid potato (*Solanum tuberosum*) is the cultivated diploid *S. stenotomum*. *Solanum stenotomum* has, however, an irregular bilabiate calyx, while the calyx of *S. tuberosum* is always regular. The irregular calyx of *S. stenotomum* is recessive to the regular calyx of the weedy diploid *S. sparsipilum*, and Hawkes⁸ has postulated that tetraploid *S. tuberosum* arose by chromosome doubling in a hybrid between *S. stenotomum* and *S. sparsipilum*. Apparent confirmation of this parentage has come from serological and electrophoretic studies¹⁹, as well as observations on polyhaploids of *S. tuberosum*²⁰, some of which have an irregular, others a regular, calyx.

Different lines of investigation sometimes suggest different conclusions. *Nicotiana tabacum* is an allotetraploid combining the S genome from *N. sylvestris* and the T genome from one of the four species in section *Tomentosae*. Geographic distribution and fertility of synthesised amphidiploids suggest the T genome donor was *N. otophora*, while morphology of the synthesised amphidip-

loids, genetic segregation ratios in hexaploid hybrids between *N. tabacum* and the T genome diploids, and chemotaxonomic studies on sterol composition and isozyme variation all implicate *N. tomentosiformis* rather than *N. otophora*^{21,22}. A possible explanation of these discrepancies is that the T genome of *N. tabacum* was not derived from any of the extant species but from an extinct progenitor common to both *N. tomentosiformis* and *N. otophora*, though this raises a further problem since *N. tabacum* is unknown in the wild and is usually assumed to have originated in cultivation, that is, fairly recently on the evolutionary time scale.

The question of multiple origins arises again in polyploid crops. If their ancestral diploids were widespread, hybridisation, chromosome doubling and successful establishment of the resultant polyploids could have occurred more than once and in more than one place. For example, a polyphyletic origin has been postulated, on chemotaxonomic grounds, for the tetraploid New World cottons. Johnson²³ argued, from seed protein electrophoretic patterns, that *Gossypium hirsutum* arose from hybridisation between an AA diploid and two different DD diploids, while studies of floral pigments²⁴ may imply that different DD diploids were involved in the origins of *G. hirsutum* and *G. barbadense* respectively. These two interpretations are in part mutually contradictory, and do not agree with cytogenetic evidence that the D genomes of *G. hirsutum* and *G. barbadense* are identical²⁵. Nevertheless, polyphyletic origins must be borne in mind when polyploid crops are considered.

Taxonomy and the domestication and dispersal of cultivated plants

Although the archaeological record potentially provides the most conclusive evidence on where a particular crop was domesticated, this record is so incomplete that inferences often have to be drawn instead from the present distribution of the crop and its wild relatives. Criteria usually used to determine the species most closely related to a crop are morphological similarity and ability to

hybridise with the crop. However, in some groups, such as *Manihot*, speciation has not been accompanied by reproductive isolation so this latter criterion is useless. Rogers and Appan²⁶ resorted to numerical taxonomic analyses in an attempt to determine where and from which species cultivated manioc (*M. esculenta*) was derived. They found that species morphologically close to *M. esculenta* occur in both Middle and South America, so were unable to decide in which continent *M. esculenta* arose.

Improved taxonomic understanding may radically alter views on the wild relatives and hence place of origin of particular cultivated plants. The coconut (*Cocos nucifera*)²⁷ was once thought to have originated in the New World because this was where the other species of *Cocos* occurred. Now, however, *Cocos* is treated as a monotypic genus whose closest living relative is African. This, together with the fossil record of the coconut and its variability and range of uses in south-east Asia, suggests that the coconut originated in the western Pacific and spread west to east, not east to west, across the ocean. The bottle gourd (*Lagenaria siceraria*) is another pantropical cultivated plant unknown in the wild and considered for a long time to be the only species in its genus. Now, however, two African genera have been merged with *Lagenaria*²⁸, in part because they readily give fertile hybrids with the bottle gourd, and it now seems that *L. siceraria* itself may have originated in Africa.

Both coconut and bottle gourd have figured largely in arguments about prehistoric human trans-oceanic voyages. Archaeologists and geographers tend to be struck by the coincidence of similar uses of similar plants on different sides of an ocean; botanists, aware that these same disjunct distributions are shared by various wild plants which are no conceivable use to man, are not found in man-disturbed habitats, and are hence most unlikely to have been spread either accidentally or intentionally by man, are usually less impressed. Seventy-four genera, including 108 species, are common to tropical America and tropical West Africa; 94 genera occur in both tropical South America and tropical Asia-Australasia²⁹. Many of the species involved are far less well-adapted for natural long-distance dispersal than the coconut or the bottle gourd.

Centres of diversity and centres of domestication

Vavilov³⁰ employed the argument that centre of diversity is a guide to centre of domestication extensively in his studies on the origin of cultivated plants, though he was careful to distinguish primary centres, where diversity is a consequence of ancient cultivation, from secondary centres, where diversity may be a consequence of hybridisation. Later critics of Vavilov's work have suggested that ecological diversity³¹, farming practice³², human migration patterns³³ and/or the breeding system of the crop³⁴ could likewise produce localised centres of diversity, in which diversity would however be unrelated to the length of time for which the crop had been cultivated. Some cultivated plants, such as the bottle gourd, may have no centre of diversity, but were probably domesticated repeatedly throughout the range of their wild progenitors³⁵. Despite the availability of appropriate techniques—notably isozyme analysis—for comparative studies of variation, and despite the interest among those concerned with conservation of crop genetic resources in knowing more about distribution of variability within crops as a guide to exploration and sampling, there have been few thorough biosystematic investigations of cultivated plants inside and outside the range of their wild relatives^{36,37} and it remains difficult to determine how generally applicable Vavilov's criteria are for determining the place of domestication of a cultivated plant.

Maize is a classic example of a crop in which variability may give misleading evidence on place of domestication. The centre of diversity of maize is in Peru, yet archaeological evidence and distribution of wild relatives demonstrate conclusively that maize was domesticated in Mexico³⁸. In tomato also the centre of diversity of the cultigen (Mexico) and the distribution of related wild species (western South America) do not coincide. Here archaeological, historical and linguistic data indicate that Mexico was the centre of domestication³⁹.

Taxonomy and the archaeological record

Interpretation of the archaeological record of cultivated plants is very much the province of taxonomy. The first step is identification and, because seeds and other hard plant parts are more likely to be preserved than fleshy fruits, flowers or unignified vegetative organs, identification usually cannot be based on traditional taxonomic diagnostic characters but has to rely instead on such characters as are available. Scanning electron microscope studies of surface features of carrot mericarps from late, possibly post-Spanish, levels of a site in coastal Peru showed that these fruits belonged to one of the indigenous New World wild species, not the introduced Mediterranean *Daucus carota* (B.P., unpublished, and S. Okeke, personal communication). Similarly, scanning electron microscope studies of starch grains and vascular tissue, particularly xylem, seem to offer the best hope of identifying various starchy tubers and roots from other Peruvian sites⁴⁰. Phytoliths (siliceous bodies in the epidermal cells of many grasses, whose shape may differ in different genera) may be preserved archaeologically when most other plant parts have decayed and attempts are being made to use phytoliths to trace the spread of maize cultivation from Mexico to South America (D. M. Pearsall, papers delivered at the 41st and 42nd Annual Meetings of the Society for American Archaeology, 1976 and 1977), through humid tropical regions where archaeobotanical preservation is generally poor.

After identification, the next step is to decide whether the archaeobotanical material comes from wild or domesticated plants. Sometimes domestication has been accompanied by morphological changes that can be recognised in archaeological specimens. In *Phaseolus vulgaris*⁴¹, both the parchment layer and the fibrous thickening of the sutures of the pods have been reduced, producing tardily dehiscent or indehiscent pods in the cultigens. The structure of the seed coat has also altered so that seeds of domesticated beans are more permeable, hence germinate more rapidly. In chili peppers⁴², natural mechanisms for seed dispersal have likewise been lost, and fruits of domesticated peppers are retained on the peduncle at maturity.

In many crops domestication has not been accompanied by qualitative changes of a sort that could be detected archaeologically. Quantitative changes in size have been extensively used in studies of animal domestication but are treated more cautiously by botanists. Exploitation, cultivation and domestication of avocados have been distinguished at different periods of a long archaeological sequence from Mexico⁴³, but this interpretation has been criticised because both parameters used (numbers of seeds, and size of largest seed) are themselves in part related to time⁴⁴. Archaeological fruits of sunflower, *Chenopodium* and *Iva* from sites in eastern North America which antedate the spread of maize agriculture from Mexico are larger than fruits of modern weedy forms. Those who do not believe that agriculture was invented independently by the Eastern Indians have argued that growth on nutrient-rich soils such as middens, selective harvesting and/or post-harvest processing could lead to fruits recovered from archaeological contexts being outside the size range of modern wild fruits; proponents of the hypothesis have pointed out that other weedy plants, such as *Phalaris*, which were collected but not cultivated, do not have larger fruits than modern forms⁴⁵. *Iva* has in addition been found archaeologically east of its present range as a wild plant, suggesting that man was involved in the spread of this species⁴⁶.

Agricultural origins in the Americas

De Candolle¹ considered that agriculture had originated independently in three regions: China; the Middle East and Egypt; and tropical America. Vavilov³⁰ recognised eight centres of origin of cultivated plants, including two in the New World. More recent taxonomic studies of New World cultivated plants and collaboration between taxonomists and archaeologists in interpreting evidence on their domestication, evolution and spread suggest that agriculture may have developed essentially independently in four different parts of the Americas. In Mesoamerica and the Andes the first unequivocal domesticates appear about the same

time, 5500–5000 BC (ref. 46), but the plants cultivated in these two centres appear in a different sequence and often belong to different species. Later some crops, notably maize, were exchanged between the two areas, but the introduced crops were absorbed into the indigenous agricultural complexes without altering the essentially distinctive character of the two regions. A third complex of crops was domesticated in the humid lowland tropics, but it is difficult to determine how early agriculture began here because conditions seldom favour preservation of identifiable archaeobotanical materials. Lowland domesticates which could survive in semi-arid conditions (manioc, sweet potato, peanut, pineapple) spread to the Mesoamerican and Andean centres, while crops which could adapt to high rainfall (maize, cotton) spread down into the jungle lowlands. In eastern North America a fourth complex of cultivated plants may have developed, but this was almost completely supplanted by the northwards spread of the Mesoamerican domesticates.

Multiple origins thus seem likely for New World agriculture in general as well as for most of those New World crops which include more than one species. Further and more critical taxonomic and archaeological work may well demonstrate that even single-species crops may have been domesticated repeatedly within the range of their wild progenitors. Domestication of cultivated plants no longer seems to be as unique and difficult a process as was once thought.

- ¹ de Candolle, A. *Origin of Cultivated Plants* (2nd edn 1886, reprinted Hafner Publishing Co., London, 1959).
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articles

Oceanic residence times of trace metals

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A detailed study of suspended matter and metal fluxes in a large marginal sea has enabled us to assess the impact of nearshore precipitation of the dissolved and particulate constituents of river discharge upon the oceanic residence times of seven trace metals. A distinct improvement in the agreement between stream flow and sedimentation rate estimates of residence time has been achieved and we conclude that most previous determinations, particularly in the cases of Fe and Mn, have been overestimated. Our calculations yield oceanic residence times of Fe which are compatible with direct measurements of the settling rate of Fe in the deep ocean.

THE oceanic residence times of trace metals have most commonly been estimated by determining either the rates of metal injection to the ocean from rivers (the stream-flow method) or the rates of metal precipitation into oceanic sediments. In a majority of cases, the metal concentrations used in these calculations to determine

the standing stock of metals in the ocean have not been the most recent and reliable estimates. This has generally led to overestimates of residence time irrespective of which method has been used. In addition, estimates based on stream-flow have largely neglected both nearshore precipitation of metals and the discharge of metals to the ocean in riverborne suspended particulate material. Obviously, for metals that are predominantly in solution in rivers, and which do not undergo appreciable precipitation in estuaries, neither of these considerations will seriously alter the calculated residence times. For elements such as Fe, however, which significantly precipitate in estuaries^{1–4}, residence times calculated without accounting for this removal will be underestimated. Conversely, although most riverborne particulates undoubtedly precipitate in the nearshore zone, the residual particulate flux will contribute large amounts of some metals, for example, Fe and Mn, to the ocean. If these fluxes are neglected, stream flow residence times will be overestimated.

It may be argued that such biases would be revealed by a comparison of the rate of incorporation in sediments with the rate

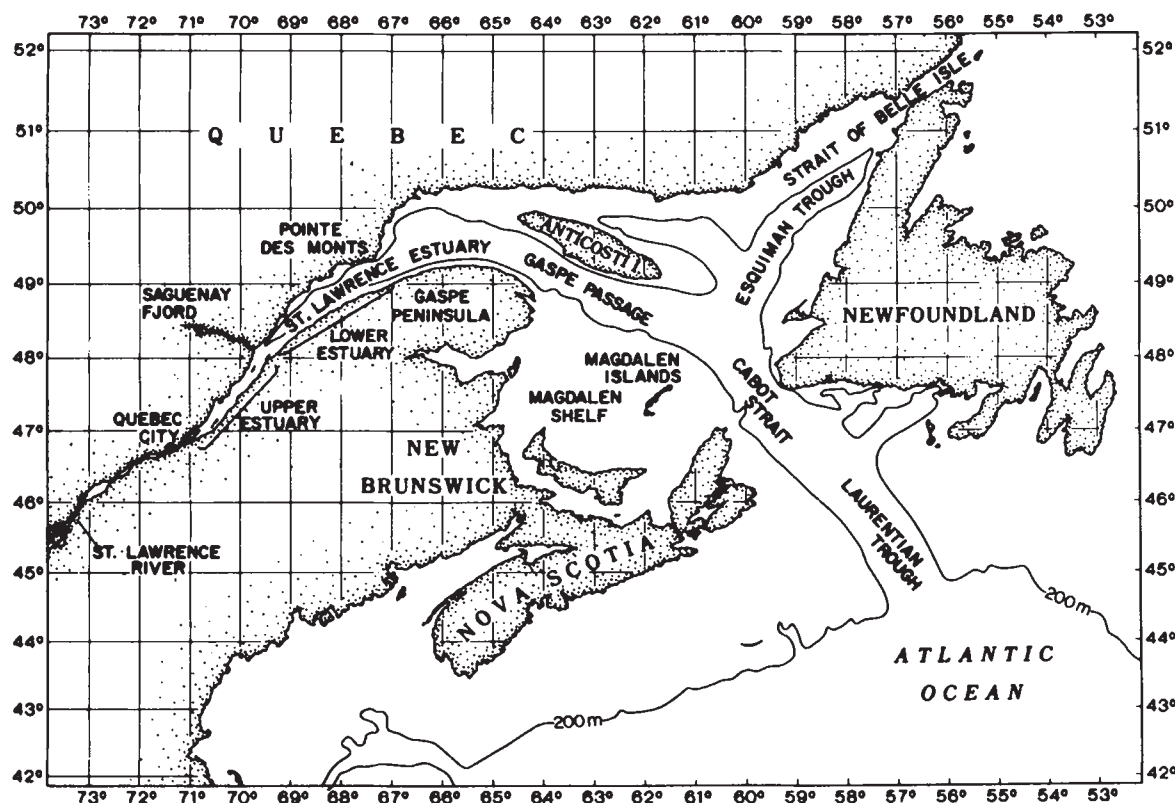


Fig. 1 Estuary and Gulf of St Lawrence.

of injection from rivers. We seek to show that this is indeed the case, and that taking into account (1) the extent of nearshore metal precipitation and (2) the particulate, as well as the dissolved, river discharges of each metal, results in improved agreement between stream flow and sedimentation rate estimates of oceanic residence times. The use of recent values for oceanic trace metal concentrations should also improve the accuracy of these estimates.

This paper is based on data collected on chemical oceanographic surveys of the St Lawrence river, estuary and Gulf of St Lawrence during the period 1972–1976. The estuary and Gulf of St Lawrence (Fig. 1) comprise a large marginal sea on the eastern seaboard of North America. Of the freshwater input to the entire system, 71% is derived from the St Lawrence River which is the eleventh largest world river in terms of water discharge⁵. We have determined the concentrations of suspended particulate matter (SPM) and seven metals in inflowing fresh and saline waters and outflowing mixed water at the two extremities of the system (Quebec City and Cabot Strait) and at two other sections in the estuarine region (near the entrance to the Saguenay Fjord and at Pointe des Monts). Nuclepore filters (0.4 μ m pore size) were used for the gravimetric determination of SPM concentrations and the metals were analysed in unfiltered samples (Fe, Mn, Co, Ni, Cu, Zn, Cd) and filtered samples (Fe, Mn) by flameless atomic absorption techniques. The resultant data have been used in a mass and salt conservation model (Fig. 2) to calculate the fluxes of each metal flowing into and out of three sequential regions of the entire marginal sea (Table 1). Since these data probably only pertain to the instantaneous fluxes at the time of sample collection, it would be unrealistic to use them for estimating the integrated annual fluxes at each section. We have only assumed, therefore, that the proportions of each component precipitated, or otherwise removed, within each region of the system remain unaltered as stream flows change with season. It has also been assumed that direct atmospheric precipitation of particulates and trace metals into the system is negligible and that exchanges through the Strait of Belle Isle, which, for water, are about 10% of those at Cabot Strait, can be ignored. In the former respect, particulate atmos-

pheric deposition has been estimated to be about 1% of the overall freshwater particulate input⁶. Finally, since the St Lawrence River is unlikely to be the sole source of sediments within the system, we have assumed that, for each metal, the percentage losses from all freshwater and marine sources are identical. As an independent assessment of the validity of the calculation we also compare in Table 1 the concentration of each metal in the internally precipitating material with the composition of pelitic sediments within the Gulf. The comparison is surprisingly good. Similarly, the calculated sedimentation rate of 415 kg s⁻¹ (1.3×10^7 t yr⁻¹) agrees well with Sundby's⁶ estimate (1.0×10^7 t yr⁻¹) of the rate at which fine-grained sediments have accumulated during the present circulation regime.

The results of scaling the St Lawrence data to the global situation are illustrated in Table 2. We first determined the weighted average composition of St Lawrence River water based

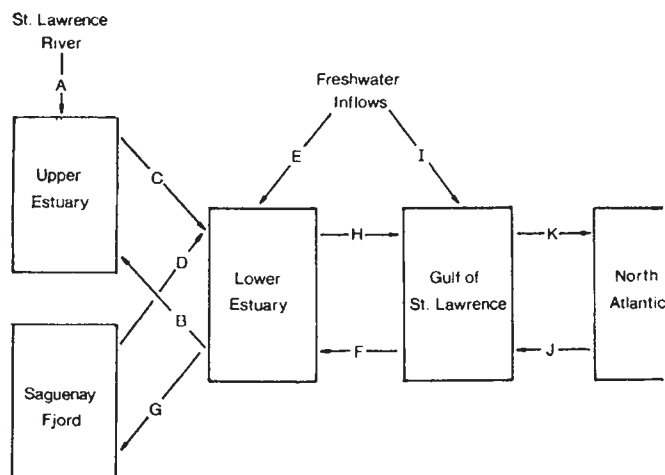


Fig. 2 Model of the St Lawrence system.

Table 1 Fluxes of suspended matter and metals through the St Lawrence system

Region	Flux	Units	Water	SPM	Fe _d	Fe _p	Mn _d	Mn _p	Co _i	Ni _i	Cu _i	Zn _i	Cd _i
Upper Estuary	A St Lawrence River inflow	kg s ⁻¹	2.1 10 ⁷	336	1.16	24.0	0.202	0.407	0.0085	0.040	0.105	0.162	0.0074
	B Saline counter-current inflow	kg s ⁻¹	5.6 10 ⁷	120	0.78	10.3	0.088	0.139	0.0050	0.040	0.045	0.082	0.0126
	C Mixed outflow	kg s ⁻¹	7.7 10 ⁷	454	1.37	30.4	0.333	0.3405	0.0098	0.090	0.114	0.137	0.0190
	A+B-C Loss	kg s ⁻¹	—	2	0.57	3.9	-0.043	0.2055	0.0037	-0.010	0.036	0.107	0.0010
Lower Estuary	D Saguenay Fjord inflow	kg s ⁻¹	4.4 10 ⁶	26.1	0.31	2.59	0.048	0.026	0.0004	0.003	0.004	0.009	0.0006
	E Additional freshwater inflow	kg s ⁻¹	2.7 10 ⁶	27.0	0.14	2.57	0.027	0.054	0.0008	0.004	0.011	0.027	0.0008
	F Counter-current inflow	kg s ⁻¹	9.24 10 ⁷	20.5	0.345	0.825	0.060	0.011	0.0018	0.032	0.059	0.196	0.0117
	G Saguenay Fjord inflow	kg s ⁻¹	2.2 10 ⁶	5	0.03	0.3	0.004	0.005	0.0002	0.001	0.001	0.003	0.0005
	H Mixed outflow	kg s ⁻¹	1.18 10 ⁸	248	0.95	14.08	0.324	0.175	0.0049	0.065	0.130	0.291	0.0150
	(C+D+E+F) - (B+G+H) Loss	kg s ⁻¹	—	154	0.40	11.71	0.052	0.112	0.0027	0.023	0.012	-0.007	0.0040
Gulf	I Additional freshwater inflow	kg s ⁻¹	3.7 10 ⁶	37	0.185	3.515	0.037	0.074	0.0011	0.006	0.015	0.038	0.0011
	J Counter-current inflow	kg s ⁻¹	3.82 10 ⁸	19.4	0.930	1.526	0.148	0.0454	0.0131	0.171	0.223	0.581	0.0194
	K Mixed outflow	kg s ⁻¹	4.12 10 ⁸	24.5	1.24	2.320	0.499	0.0603	0.0177	0.197	0.317	0.808	0.0245
	(H+I+J) - (F+K) Loss	kg s ⁻¹	—	259	0.48	15.98	-0.050	0.223	-0.0004	0.013	-0.008	-0.094	-0.0007
Sum of losses			kg s ⁻¹	415	1.45	31.59	-0.041	0.541	0.0060	0.026	0.040	0.006	0.0043
Whole System	Metal concentration in internally sedimenting material	%	—	—	8.0	—	0.13	—	0.0015	0.0063	0.0097	0.0015	0.0010
	Metal concentration in Gulf of St Lawrence pelitic sediments (from refs 18 and 19)	%	—	—	6.0	—	0.10	—	0.0016	0.0033	0.0025	0.0100	0.00002
	Proportion of total inputs lost internally	%	—	93	89	—	47	—	25	12	11	0.1	15

Subscripts d, p and t denote dissolved, particulate and total metal respectively.

Characterisation of water flows in Cabot Strait and Pte. des Monts sections are based upon the geostrophic calculations of El-Sabh²⁰

on duplicate samples collected at two stations on a section across the river at Quebec City for the months April to September inclusive. We then calculated the resulting river-derived annual fluxes through Cabot Strait by correcting for the deposition of the river constituents within the entire marginal sea. Estimates of the particulate fraction of each metal in the Cabot Strait outflow were then used to split the total fluxes into dissolved and particulate components. For Fe and Mn, these estimates are based upon experimental determinations while others are calculated from average terrigenous rock composition and the concentrations of total metal and suspended matter in the outflow. The dissolved and particulate metal fluxes have then been scaled up to the world river discharge on the assumption that the losses of river-derived material in the Gulf of St Lawrence are typical of those in the nearshore receiving waters of all world rivers. The scaling has been achieved by using Holeman's⁷ figure for the particulate material discharge via rivers to scale the fluxes of particulate metal and Livingstone's⁸ value for the world river water runoff to scale the fluxes of dissolved metal. Implicit in the former scaling process is the assumption that the composition of particulate material discharged by other rivers is similar to that found in the case of the St Lawrence. Since the composition of weathered terrigenous rock is not greatly variant, especially in the cases of Fe and Mn, this assumption is not unreasonable. Similarly implicit in the dissolved metal scaling process is the assumption that the dissolved composition of St Lawrence River water is typical of that of world rivers. Obviously, the validity of this assumption must await more accurate estimates of the composition of other major river waters

but the values we have used are well within the range of commonly accepted values for other fresh water discharges.

Having carried out this scaling process it is a simple matter to recombine the dissolved and particulate metal fractions and calculate the oceanic residence times using 1.32×10^{21} l as the volume of water within the oceanic basins and the average concentration of each metal in oceanic waters shown in Table 2, after Bewers *et al.*⁹. Comparisons between these latter concentrations and other previously reported values are made in this reference. The agreement with values reported more recently¹⁰⁻¹² is good. The results of such a calculation and a comparison with residence time estimates based on those of Goldberg and Arrenhius¹³ but using the recent oceanic metal concentrations are also shown in Table 2.

We have not attempted to calculate the residence time of particulate material since this requires a better knowledge of the mean concentration of purely detrital material in the deep ocean. Nevertheless, we have included in Table 2 an estimate of the suspended particulate influx to the ocean although we realise that its acceptance implies that there is no substantial export of internally generated biogenic particles through Cabot Strait. If the terrigenous detrital component of particulate material is diluted with internally generated biogenic particles the nearshore losses of suspended matter will be underestimated. Our estimates of particulate fluxes at Cabot Strait are, however, based on measurements in July 1976 at which time the composition and quantity of suspended matter in the Gulf of St Lawrence outflow indicate that the material is predominantly detrital.

Table 2 Residence time calculations

	Units	SPM	Fe	Mn	Co	Ni	Cu	Zn	Cd
Annual outflow from St Lawrence River	kg yr ⁻¹	4.4 × 10 ⁹	3.8 × 10 ⁸	1.0 × 10 ⁷	1.4 × 10 ⁵	7.6 × 10 ⁵	1.4 × 10 ⁶	2.2 × 10 ⁶	1.2 × 10 ⁵
River flux corrected for nearshore losses	kg yr ⁻¹	3.08 × 10 ⁸	4.18 × 10 ⁷	5.30 × 10 ⁶	1.05 × 10 ⁵	6.69 × 10 ⁵	1.25 × 10 ⁶	2.20 × 10 ⁶	1.02 × 10 ⁵
Particulate fraction at Cabot Strait	%	100	65	11	4	1.3	0.7	0.2	0.02
Global dissolved flux	kg yr ⁻¹	—	1.08 × 10 ⁹	3.48 × 10 ⁸	7.42 × 10 ⁶	4.86 × 10 ⁷	9.11 × 10 ⁷	1.62 × 10 ⁸	7.51 × 10 ⁶
Global particulate flux	kg yr ⁻¹	1.26 × 10 ¹²	1.11 × 10 ¹¹	2.39 × 10 ⁹	1.72 × 10 ⁷	3.56 × 10 ⁷	3.57 × 10 ⁷	1.80 × 10 ⁷	8.35 × 10 ⁴
Sum of global dissolved and particulate fluxes	kg yr ⁻¹	1.26 × 10 ¹²	1.12 × 10 ¹¹	2.74 × 10 ⁹	2.46 × 10 ⁷	8.42 × 10 ⁷	1.27 × 10 ⁸	1.80 × 10 ⁸	7.59 × 10 ⁶
Concentration in marine waters (ref. 9)	μg l ⁻¹	—	2.27	0.08	0.023	0.23	0.39	1.07	0.044
Oceanic residence time based on stream flow	yr	—	27	39	1200	3600	4100	7800	7700
Oceanic sedimentation rate (from ref. 13)	kg yr ⁻¹	1.5 × 10 ¹²	1.0 × 10 ¹¹	2.0 × 10 ⁹	3.9 × 10 ⁷	1.7 × 10 ⁸	8.0 × 10 ⁷	7.8 × 10 ⁷	6.3 × 10 ^{5*}
Oceanic residence time based on sedimentation rate	yr	—	30	53	780	1800	6400	18000	92000

* Assumed mean Cd concentration in ocean sediments of 0.42 mg kg⁻¹ (ref. 21)

Global dissolved flux based on scaling river water discharge to world river runoff of 3.24×10^{16} kg yr⁻¹ (ref. 8)

Global particulate flux based on scaling river particulate discharge to world river sediment discharge of 1.8×10^{13} kg yr⁻¹ (ref. 7)

Use of three significant figures in intermediate calculations is merely to avoid roundoff errors in tabulated values.

As can be seen in Table 2, the agreement between the trace metal residence times based on stream flow and sedimentation rate is, with the exception of those for Cd, quite good and certainly better than other recent comparisons^{10,14}. Furthermore, the calculated influx of fluvial suspended particulate matter is comparable with the rate of oceanic sedimentation¹³. The discrepancy between the calculated rate of cadmium input to the oceans by rivers and the rate of cadmium sedimentation is too large to be attributed solely to an abnormality in the composition of the St Lawrence River, especially in view of a recent analysis of Amazon River water¹¹. It is probable that the sedimentation rate is a more realistic estimate of oceanic throughput than river input in this case but some re-examination of both the extent of nearshore precipitation and the Cd distribution in marine sediments seems warranted. Co and Ni are the only elements for which the stream flow residence times are greater than the sedimentation rate residence times. This suggests that other forms of input are more important for these elements than the others. It is interesting that when the relative metal contents of terrigenous rock and corrected river flux are compared, Co and Ni are the only elements which are enriched in terrigenous rock. Dry atmospheric fallout may, therefore, be more important for Co and Ni than for the other elements.

Overall, our estimates of residence time are generally shorter than values given in previous compilations^{15,16}. This feature is most pronounced in the cases of Fe and Mn. Labeyrie *et al.*¹⁷ have estimated, on the basis of weapon-produced ⁵⁵Fe settling rates, that the residence time of Fe is about 10 yr. We are thus not alone in proposing such a short residence time for this element. Concern has often been expressed about the difficulty of rationalising residence times much shorter than oceanic mixing times with evidence for the relatively homogeneous distribution of the same elements in ocean sediments. This conflict will be reconciled if biological activity and horizontal dispersion/mixing of both particulate and dissolved metals is sufficient to ensure reasonably uniform metal concentrations in surface waters beyond the continental shelves. Settling of the inorganic and refractory biogenic components of the uniformly distributed particulate matter will then account for the relatively uniform distribution of the metals in the sediments. The residence time of Fe in the surface layer of the open ocean, calculated according to the method of Goldberg *et al.*¹⁶ and using data from the western North Atlantic⁹, is 16 yr compared with 20 yr for water. Mn, unlike most other metals, has a concentration in the surface layer which exceeds that

in deep waters⁹. This suggests that its surface layer residence time is comparable with that of water. Thus it is likely that both these metals will exhibit fairly uniform distributions in open ocean surface waters despite their short oceanic residence times. The overall residence time of Fe corresponds to a settling rate of $4 \times 10^{-6} \text{ m s}^{-1}$ compared with 10^{-5} m s^{-1} for particles containing ⁵⁵Fe (ref. 17). The equivalent Stokes particle size for inorganic detrital material is about 3 μm . Nevertheless, the primary host for downward transport of Fe in the open ocean is likely to be larger, lower density, biogenic particles, such as faecal pellets, with similar settling velocities.

Metals which remain predominantly in solution are removed from the water column considerably more slowly. Their distributions in the surface layer are probably controlled by mixing and metal incorporation into the biological components that are largely regenerated in the mixed layer. The deep water dissolved metal concentrations will be maintained by the degradation and dissolution of biogenic detritus and the balancing accumulation of metals on to exposed inorganic particle surfaces in the water column and in the sediments. The residence times of the predominantly dissolved metals, therefore, probably represent the time scales in which organic scavenging, biogenic particle settling and dissolution, and final association with inorganic particulate surfaces in the deep water are occurring.

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DNA arrangement in isometric phage heads

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DNA is wound tightly into phage heads in such a way that it tends to form layers concentric with the rigid protein shell. In P22 and wild-type lambda, DNA completely fills the internal volume, with a highly uniform local packing of adjacent segments; in lambda deletion mutants containing less than a full genome, the local packing distance increases correspondingly.

DETERMINING the packing geometry of nucleic acid in virus particles presents a rather difficult experimental problem.

In the heads of double-stranded DNA-containing bacteriophage, such as λ or P22, the nucleic acid is condensed 250-fold, and DNA strands are then so closely packed that conventional electron-microscopic techniques have yielded no interpretable images. Moreover, such phages are too large for application of the X-ray diffraction methods used to visualise single-stranded RNA in tobacco mosaic¹ and tomato bushy stunt viruses². A large number of indirect approaches have been tried (see ref. 6), but virtually the only direct data bearing on the problem of DNA arrangement are those of Richards *et al.*³, who managed to visualise gently disrupted bacteriophage particles in the electron microscope. They concluded that DNA

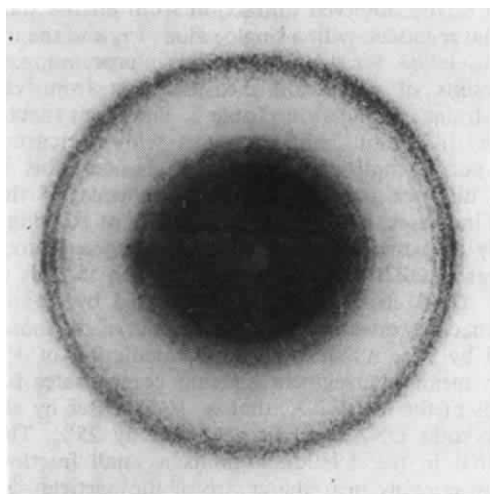


Fig. 1 X-ray diffraction from phage P22. The outer set of rings corresponds to the 25 Å diffraction discussed in the text and shown as a scan in Fig. 2. The experimental conditions are described in ref. 4.

is coiled into the phage head—either coaxially like thread on a spool or with random direction like a ball of yarn. Recent small-angle X-ray scattering studies of intermediates in the P22 head-assembly pathway led us to recognise evidence for considerable long-range order in packaged DNA⁴. We describe here an extension of that work, leading to some conclusions about the organisation of DNA in bacteriophages with isometric heads—P22, λ and T7.

Diffraction from phage

A photograph of X-ray diffraction from a solution of phage P22 is shown in Fig. 1. The series of rings at small angles can be interpreted in terms of the radial distribution of density in the particle⁴. They show that in first approximation, the P22 particle diffracts like a uniformly filled sphere. The strong outer band, corresponding to a spacing of about 25 Å in the structure, is due to DNA^{4,5}. A densitometer trace of the intensity in this band is shown in Fig. 2. The diffraction is modulated by a series of ripples, having a periodicity approximately equal to the reciprocal of the head diameter. Experiments with other isometric phages (various strains of λ , T7) show this modulation to be not a mere superposition of other diffraction but rather a consequence of the geometry of DNA packing. Details of the interpretation of these observations are presented below. At wider angles, the diffraction from P22 and λ phage heads show the characteristic B-form DNA pattern, with maxima near 12, 8 and 3.4 Å (ref. 4).

Organisation of phage DNA

The diffraction from phage DNA *in situ*, just described, reveals both local and long-range order in the phage head. The presence of local order, indicated by the strong 25-Å diffraction, has been recognised for some time^{6,8}; the long-range order, indicated by the rippled modulation of this diffraction, was observed more recently by Earnshaw *et al.*⁴, who first examined isometric phage with X-ray cameras of sufficient resolution.

Local order: adjacent segments of the DNA molecule are packed in a locally parallel array, with a side-to-side spacing that can be determined from the position of the 25-Å diffraction band⁴. From the width of this band, we can conclude that this spacing is quite uniform throughout the phage head with an 'apparent domain size' of ~ 125 Å (refs 4, 5, 16). Assuming hexagonal packing, as in concentrated gels of DNA *in vitro*⁷, the interhelix distance is equal to 1.16δ where δ (~ 25 Å) is the spacing of the observed diffraction (Fig. 4 and Table 1, where the precise determination of δ from the pattern is described). We can also calculate the area of the local 'unit cell' (Fig. 4)—the cross-sectional area occupied by one DNA strand—this area, multiplied by the length of the P22 DNA molecules, gives the volume, V , taken up by the packed DNA. This calculated volume is in fact equal to the internal volume of the wild-type phage head, accurately determined from the structure of empty heads⁴. These calculations are summarised in the top line of Table 1.

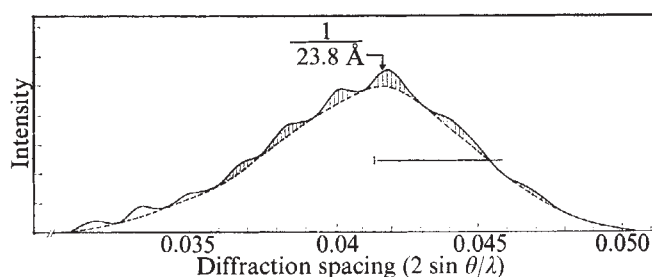


Fig. 2 Densitometer trace in the 25-Å region of a film similar to the one shown in Fig. 1. The film was scanned as described in ref. 4. The shaded peaks are those used for fitting the model in Fig. 3. The horizontal bar indicates the half-width at half-height, 0.004 Å^{-1} . The data have been corrected by subtracting a smooth background due to diffuse scatter of the X-ray beam.

Long-range order: the ripples that modulate the intensity of the 25-Å diffraction have a wavelength of about $1/540 \text{ Å}$, that is, approximately the reciprocal of the internal diameter of the phage head. This indicates coherent diffraction from opposite sides of a particle: locally ordered DNA segments on one side must be diffracting with a fixed phase relative to locally ordered segments on the other. A linear DNA molecule

Table 1 Measured and calculated dimensions for DNA packing in phage heads*

Phage	d (Å)	δ (Å)	A (Å ²)	l (Å)	V (Å ³)	r (Å)	r_0 (Å)	n	f	ref.
P22	24.2	23.8	638	14.0×10^4	8.9×10^7	278	278	$4(+1)$	72	8
λ b221	(78) 26.2	25.8	772	12.6	9.75	285	294	5	90	9
λ b2	(88) 25.1	24.7	708	14.1	10.0	288	294	5	86	9
λ b515-b519	(89) 25.0	24.6	701	14.5	10.1	290	—	—	—	9
λ +	(100) 24.0	23.6	647	16.1	10.4	292	294	5	80	9
λ att ²	(105) 23.5	23.2	625	16.8	10.5	293	294	4	74	10

* d , mean Bragg spacing of DNA diffraction band measured from films; δ , interlayer spacing, derived from d by applying an approximate intensity-profile correction to account for the fact that the intensity maximum will be somewhat displaced from the reciprocal of the spacing between shells; A , area of local unit cell of DNA segment packing (cell assumed hexagonal, so that $A = 1.16\delta^2$); l , length of DNA; V , volume occupied by DNA in head ($V = Al$); r , minimum radius to which DNA must extend, calculated from $V = (4/3)\pi r^3$; r_0 , radius of DNA-protein boundary, calculated from best fit in Fig. 3 by adding 12 Å to the mean radius of the outermost DNA shell; n , number of DNA shells used for best fit to diffraction data (Fig. 3); in P22, a protein shell has also been included; f , fraction of total DNA in coherently scattering shells; ref. reference for DNA molecular weight.

The number in parenthesis after the name of the lambda phage strain indicates the percentage of wild-type DNA complement present. The genotype of λ att² was i^{21} .b515.b519.int₂.xis₄.att².bio⁺.uvrB⁺; all other λ strains were s7 (lysis-defective). Diffraction measurements were recorded from λ in 0.05 M NaCl, 0.1 M Tris-HCl (pH 7.4), 0.001 M MgCl₂ and from P22 in 0.01 M Tris-HCl (pH 7.5), 0.005 M MgCl₂.

is not likely to form a single crystal inside a spherical phage head (for example, the recent demonstration by Lerman *et al.* of the absence of chain-folding in ethanol-precipitated DNA¹⁹). Coherence across the phage particle must, therefore, be imposed by the regular, isometric protein shell within which the DNA is contained. Indeed, we would expect segments of the DNA molecule, locally parallel and tightly packed against the inner surface of the phage head, to fall into layers spaced regularly inward from the protein boundary. A spherically averaged picture of the particle will thus show distinct shells of density concentric with the protein shell itself. From the degree of local order implied by the intensity profile, we expect to see three or four such shells before packing irregularities destroy the uniformity of their diameter (Fig. 4). The same mode of packing continues at smaller radii, but packing faults become increasingly frequent.

The calculation presented in Fig. 3 shows that the ripples modulating DNA diffraction from P22 can indeed arise from a shell-like density fluctuation in the particle. The calculation assumes that n -concentric DNA layers, differing in radius by increments of δ , are packed within the protein shell. This relatively tight shell⁴ also contributes to the X-ray scattering since its inclusion in the calculation as a shell 24 Å from the outermost DNA layer improves the fit in Fig. 3. The radius of the outermost layer of DNA, 266 Å, has been chosen to achieve the best fit to the observed intensity profile. The radius of the boundary between DNA and protein, denoted r_0 in Fig. 4 and Table 1, can then be taken as this shell radius plus 12 Å (an estimate of the ionic radius of the DNA cylinder, see atomic coordinates in ref. 7). The parameters that best fit the intensity profile are $r_0 = 278$ Å and $n = 4$, where $\delta = 23.8$ Å has been determined directly from the mean spacing of the DNA diffraction band. This value of r_0 is, in fact, the same as the radius corresponding to V (the calculated total DNA volume). Likewise, it is in accord with the inner radius of the protein shell, determined by small-angle X-ray scattering from empty heads⁴.

The trace in Fig. 2 shows that the ripples modulating the 25-Å diffraction band account for about 15% of the total intensity. Since only one of the three (100) planes in the local hexagonal lattice (marked by arrows in the insert in Fig. 4) will contribute to this sampled diffraction, we expect the ripples to account for a maximum of one-third of the integrated intensity (actually somewhat less, since the outer layers account for only about 75% of the DNA molecule). Irregularities in the lattice and layer spacing will fill in the minima between ripples, so that the observed ratio of sampled to diffuse intensity is consistent with the picture drawn in Fig. 4. Note, that the envelope of the rippled intensity follows the diffuse diffraction: the local order is essentially the same in all directions, but the packing boundary imposed by a rigid head leads to a long-range radial regularity.

Deletion mutants of phage λ

We have interpreted the diffraction from P22 phage DNA *in situ* in terms of locally parallel segments of DNA, packed tightly into the head in such a way that the polymer tends to form layers concentric with the outer protein shell. This model is summarised diagrammatically in Fig. 4. Further evidence for such a picture comes from measurements on a series of deletion mutants of bacteriophage λ . Unlike P22, which packages a constant headful of DNA, λ packages only the DNA between two distinct sites on the viral chromosome. As a result, genetic deletions result in production of phage heads containing less DNA. We have, in this way, obtained diffraction from structures with varying amounts of DNA packed into a head of constant size. The parameters of the model described above for P22 should be determined in a uniform way for the entire series of measurements on λ deletions: r_0 should be the same for all these structures, since it is in effect determined by the inner radius of the protein shell, and d should be given by the mean spacing of the DNA diffraction band, as before. Our

ability to fit the observed diffraction from all the stains by a simple n -layer model, with a single value of r_0 and the measured δ for each deletion, is a strong test of this interpretation.

The results of diffraction measurements from various λ deletion strains are shown in Table 1. The mean spacing, d , of the DNA diffraction band increases with decreasing DNA content, but its half-width does not change. This indicates that the distance between adjacent segments of the DNA molecule increases, but that the uniformity of packing remains essentially the same. In other words, the degree of local order is as great in b221 as in wild-type λ , even though the total length of DNA in the head has decreased by 22% and the average spacing between adjacent segments of the molecule has increased by 9%. As shown by the calculation of V , the increase in mean intersegment spacing compensates for about two-thirds of the lost DNA, that is, V decreases by about 7% when the total DNA content decreases by 25%. This result implies that in the deletion mutants a small fraction of the DNA—presumably near the centre of the particle—is packed more loosely than the rest.

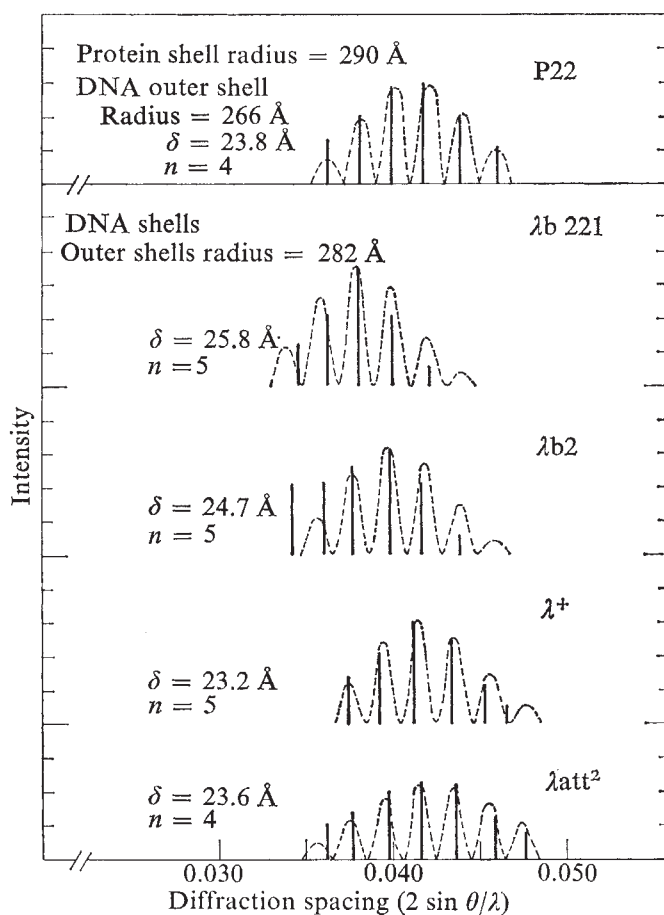


Fig. 3 Correspondence of model calculations to observed diffraction for phage 22 and various λ mutants. Spikes represent position and scaled height of intensity maxima (shaded ripples in Fig. 2); — — — shows scattered intensity calculated for a series of concentric shells¹⁸, with each shell weighted according to the amount of scattering material it contains. The calculated intensity equals F^2 , where

$$F = \sum_{i=1}^m 4\pi r_i^2 \left(\frac{\sin 2\pi r_i s}{2\pi r_i s} \right)$$

and r_i is the radius of the i th shell (m in all) and $s = 2 \sin \theta / \lambda$, the diffraction spacing. There are n shells of DNA spaced δ Å apart, and in the case of P22 a shell for protein has also been included. For λ mutants, where head size is constant, we have assumed a fixed radius of 282 Å for the outer DNA shell: to obtain r_0 , the DNA-protein boundary, 12 Å must be added to this figure.

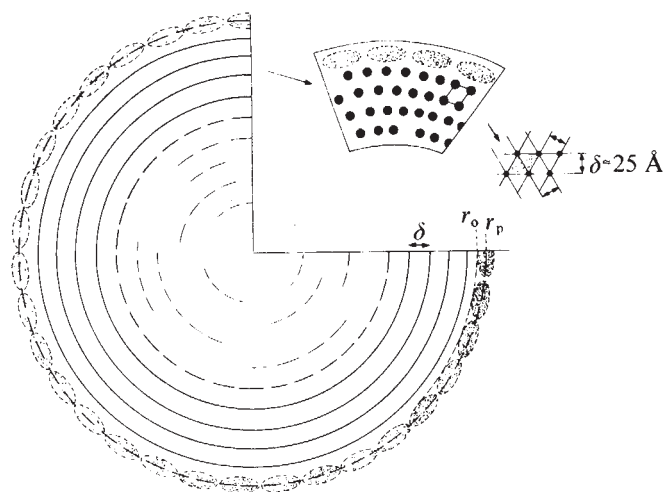


Fig. 4 Diagrammatic representation of the model used to obtain curves in Fig. 3. The protein (radius = r_p) is shown stippled; the radius of the DNA-protein boundary is r_0 ; the inter-shell spacing is δ . Adjacent segments of the DNA helix are parallel, with the local unit cell assumed to be hexagonal^{7,10}. If this is the case, packing faults must be introduced in inner shells, as shown. For this reason, the innermost DNA is shown as partially organised shells, though it could be packed in some other manner. For phage P22 the model includes a shell at radius r_p and four inner shells with spacing δ . For the λ mutants the model includes five shells with spacing δ , the outer shell at a radius of 282 Å.

All strains studied show the same rippled modulation of the DNA diffraction described above for P22. The spacing between these ripples remains essentially constant with varying DNA content, but the positions of the maxima and minima shift, as indicated in Fig. 3. The results in Table 1 and Fig. 3 show that we can fit all observations by models with four or five coherently scattering layers, without changing r_0 . That is, we can imagine an outer layer of DNA segments, packed tightly against a protein shell of fixed size, and successive layers of segments packed against the first with a spacing that increases with decreasing DNA content. Moreover, the best value of r_0 for all strains agrees well with the inner radius of urea-treated λ -petit λ , an *in vitro* analogue of empty heads (Earnshaw and Hendrix, in preparation). In contrast with P22, the protein shell itself does not appear to contribute to the 25 Å diffraction, and it has not been included in the model calculation.

The fraction of the DNA molecule that would be contained in these coherently-diffracting layers varies from about 90% for b221 to about 75% for att² (Table 1). In all cases, then, a large fraction of the molecule is packed in this way. There are probably several further layers, but at this distance from the constraining walls of the phage head, the segments would be sufficiently out of register that they would not contribute to ripples in the 25-Å diffraction.

The results from λ strains in Table 1 refer to phage in 0.05 M NaCl, 0.01 M Tris (pH 7.4), 0.001 M MgCl₂. Substitution of 0.01 M polyamine (spermidine or putrescine) for Mg²⁺ has no effect on the measured spacings. Moreover, diffraction from intracellular λ b2.s7 accumulating in unlysed su⁻ cells shows the 25 Å band quite clearly. We conclude, as we did earlier for P22⁴, that the arrangement of DNA in a phage head does not change when lysis alters its ionic environment. Polyamine may, however, be important for the initial packaging event¹¹.

Three dimensions

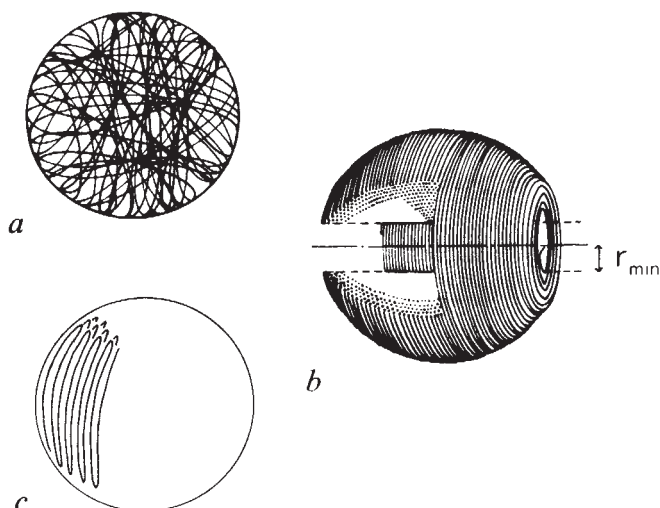
What more detailed pictures of DNA packing are consistent with the picture presented above? The electron micrographs of gently-disrupted phage heads obtained by Richards *et al.*³ show unexpanded DNA bundles, flattened into the grid: DNA

strands near the periphery of these bundles always have a circumferential orientation. These micrographs suggest that DNA is not folded with sharp kinks inside the phage head, but rather, that it is wound with relatively uniform curvature. Richards *et al.*³ proposed two limiting descriptions of DNA in phage heads based on these images: a 'ball of string' and a 'concentric spool'. In the first (Fig. 5a), DNA is wound roughly along a great circle path at any radius, with no particular relation of the tilt of the planes of successive great circular windings. In the second (Fig. 5b), the molecular windings are concentric with a single axis. Our observations seem to be inconsistent with the ball-of-string picture. The crossing of successive turns in a ball of string does not lead to parallel adjacent strands or to radially concentric layers. Such crossing also leaves gaps too large to be compatible with the tight packing of DNA in the head. Coaxial winding does not present these difficulties: segments are locally parallel and closely packed. Our data do not specify the relative orientation of these windings and the axis of the phage tail.

Certain folded structures might also be consistent with an overall organisation into shells of DNA segments layered inward from the protein, provided that the folds were appropriately positioned (Fig. 5c). Folds could plausibly result only from interaction of DNA with specific sites on the inner surface of protein subunits. The continuous variation of DNA spacings observed in λ deletions is difficult to reconcile with a fixed set of folding positions. Moreover, the local symmetry of the protein surface lattice implies that only radially-directed DNA can have an equivalent orientation with respect to each folding site: the uniformity of packing we have observed rules out a radial direction. Finally, we would not expect folded structures, when flattened on to an electron microscope grid, to show the images of coiled, circumferentially-directed strands seen by Richards *et al.*³. For these reasons, we prefer the coiled structure shown in Fig. 5b.

In any structure with more or less uniformly bent DNA, the stiffness of the polymer will prevent windings of very small radius, leaving a roughly cylindrical cavity in the centre of the head (Fig. 5b). Changes in packing of DNA in this region—estimated below to be less than 10% of the total volume of the head—might account for the slight decrease in V with decreasing DNA content. This could also account for the region of low density seen in the centre of λ and λ dg in ultra-thin sections in the electron microscope¹⁸. A reasonable value for $r_{\min}$, the radius of the cylindrical cavity, is the radius of a circle of DNA for which the bending enthalpy equals ~ 7 kcal mol⁻¹—an estimate for the enthalpy of a single 180°

Fig. 5 Schematic representation of various models discussed in the text. *a* Ball-of-string. *b* Coaxial spool. *c* Chain-folded structure (with 180° folds at each end).



fold¹². The bending enthalpy of DNA was determined by Gray and Hearst¹³: their figure of $\Delta H_{\text{bend}} = 75 \text{ kcal } \text{\AA}^{-1} \text{ mol}^{-1} \text{ rad}^{-2}$ is applicable, strictly speaking, only to radii of curvature comparable with the solution persistence length ($\sim 500 \text{ \AA}$), but its use with rather smaller radii seems adequate, since even for $r = 50 \text{ \AA}$ the bend is only about 4° per base pair. With this value for ΔH_{bend} , we get $r_{\text{min}} \sim 60 \text{ \AA}$. Also, using this estimate of r_{min} , integration over the total occupied volume yields $\Delta H_{\text{tot}} = 4\pi(\Delta H_{\text{bend}}/A)(r \ln(2r_0/r_{\text{min}} - \frac{1}{2}) - r_0 + r_{\text{min}}/2) \sim 500 \text{ kcal}$ (where A and r_0 taken here as $647 \text{ \AA}^2$ and $294 \text{ \AA}$, appropriate for λ , are defined in Table 1 and Fig. 4). To fold DNA into a λ head would require more than $l/2r_0$ folds (l is the DNA length; $2r_0$, the head internal diameter)—more than 2,000 kcal. The cylindrical cavity defined by r_{min} contains about 9% of the total volume. The column V in Table 1 shows that in λ b221, only about 91% of the total volume inside $r_0 = 294 \text{ \AA}$ is required for DNA packed with spacing $\delta = 25.8 \text{ \AA}$. In this extreme deletion, then, the central regions might actually be nearly free of DNA. In other forms, a larger fraction of the total volume is required (all of it in wild type and att²). In these structures, most of the DNA can be coiled into cylindrically concentric shells, but up to 10% might be folded back and forth lengthwise in the central region (a possibility suggested to J. Schellman), perhaps passing from there to the phage tail (see cross-linking experiments with λ and other phages—refs 14 and 15).

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Hendrix (λ b221), Mark Ptashne and Andrea Jeffrey (λ b2), William McClure (λ^+ , T7), Susan Gottesman (λ att²), and Phillip Youderian (λ n515 b519). We thank Jonathan King and David Botstein for useful criticism of the manuscript. We acknowledge grant support from the National Cancer Institute (to S.C.H.) and from the National Institute of General Medical Sciences (to J. K.), an NSF Predoctoral Fellowship (to W. C. E.), and a PHS Research Career Development Award (to S.C.H.).

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Initiation of chick cell division by trypsin action at the cell surface

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Trypsin immobilised on polystyrene beads causes initiation of cell division which cannot be accounted for by trypsin released into the medium or into the cells. Also, initiation by soluble trypsin is inhibited by immobilised soybean trypsin inhibitor. These results demonstrate that trypsin can initiate proliferation at the cell surface.

THE site of action of peptide hormones and certain growth factors is often presumed to be at the cell surface. Indeed, binding studies and isolation of binding proteins by affinity chromatography have demonstrated that specific receptors for these molecules exist in the plasma membrane. Such receptors might be involved primarily in transport or other functions, however. For example, it has been shown that there are cell surface receptors for steroid hormones even though their site of action is clearly inside the cell¹. There are reports that certain peptide hormones and growth promoters can act without entering cells^{2–6}, but most of this evidence has been obtained with Sepharose-immobilised preparations. The validity of these studies has been challenged^{7–10} primarily because of the amount of soluble material released^{7,8} and because such material may be 'superactive'¹⁰. Therefore, it is still questionable whether peptide factors can exert their biological effects without entering cells.

Trypsin and certain other proteases initiate proliferation of cultured quiescent chick embryo fibroblasts (CEF) (refs 11–18). This system has two important advantages for studying

the mechanisms involved in initiation of cell proliferation. First, the initiation occurs in serum-free medium without additional growth factors¹³, and second, it is caused by purified agents with well characterised proteolytic activities. In spite of these advantages, little progress has been made towards elucidating the actual mechanisms involved. It has been reported that 5-min treatments with soluble proteases or proteases linked to Sepharose beads doubles the number of mouse 3T3 cells^{19–21}. This would suggest that trypsin acts at the cell surface. But other investigators have not achieved significant initiation of 3T3 cell division with trypsin using various experimental conditions including exposures of up to several days^{17, 22, 23}. Even in chick cells, whose division is readily stimulated by trypsin, the protease must be present for at least 2 h to cause a significant increase in cell number, and the fraction of cells which respond increases with length of exposure up to 24 h (refs 17, 18). Such lengthy exposure times suggest a more complex mechanism of initiation than the simple binding or cleavage of specific receptors. Indeed, it has not been possible to correlate cleavage of specific surface membrane proteins with initiation of proliferation by proteases^{14–16, 24}. Moreover, both thrombin (ref. 25 and B. M. Martin and J. P. Quigley, personal communication) and trypsin²⁶ accumulate inside cultured cells after exposures sufficient to initiate cell division. These considerations prompted us to develop techniques to rigorously determine if trypsin could initiate proliferation of chick fibroblasts without entering the cells.

We report here that trypsin covalently attached to poly-

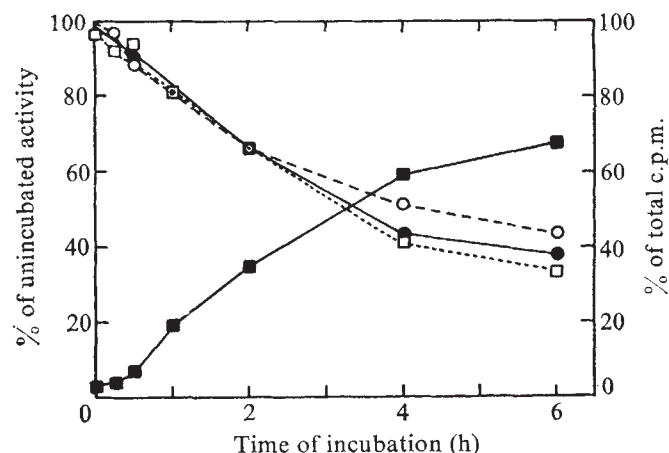


Fig. 1 Autodigestion of ^{125}I -trypsin. Correlation between TCA-precipitable radioactivity (c.p.m.), trypsin activity, and mitogenic activity. Trypsin, iodinated with Chloramine T (65,000 c.p.m. μg^{-1}), was incubated at 37°C in PBS (pH 7.4). After the indicated times, aliquots were removed and assayed for acid-soluble (■) and precipitable (□) radioactivity (5% TCA), for proteolytic activity (●) as determined by hydrolysis of *p*-toluene-sulphonyl-L-arginine methyl ester³³, and for mitogenic activity (○) on quiescent CEF as described in Table 1. When compared with unlabelled trypsin, the ^{125}I -trypsin used in these experiments retained all of its mitogenic and proteolytic activity. In addition, the loss of these activities followed the loss of TCA-precipitable radioactivity. These observations show that ^{125}I -trypsin molecules are active and representative of both labelled and unlabelled molecules.

styrene beads initiates proliferation of secondary CEF. Results from various experiments have demonstrated that this initiation is not caused by trypsin released from the beads. In addition, we report that soybean trypsin inhibitor (SBTI) attached to polystyrene beads can inhibit the proliferative effect of cell-associated trypsin even if added 4 h after the cells have been exposed to initiating levels of the protease.

Immobilised trypsin preparations

In searching for a stable form of immobilised trypsin, we first tried two commercially available preparations, trypsin-Sepharose (Worthington), and trypsin-acrylamide (Sigma). Addition of optimal amounts of either preparation to quiescent CEF resulted in population doublings after 24 h. But, a cell number increase nearly as large was achieved either with medium from which the beads had been removed, or with the beads physically separated from the cells by nylon nets during the 24 h incubation. This demonstrated that the observed initiation resulted largely from released trypsin. In contrast to the above preparations, trypsin linked by carbodiimide condensation to carboxylate-modified polystyrene beads initiated division of only about half of the cells. Medium from which these beads had been removed did not initiate CEF, however, suggesting (1), that the linkage was relatively stable and (2), that the observed initiation was due to trypsin still attached to the beads. We therefore used polystyrene bead preparations for all of the following experiments.

Active component of polystyrene-immobilised trypsin

Table 1 shows results from a representative experiment which demonstrated that initiation of CEF by trypsin-beads was due to trypsin and not the polystyrene beads or a 'nonspecific' protein-bead complex. Addition of $50\text{ }\mu\text{g}$ of trypsin-beads (~ 225 beads per cell) to quiescent fibroblasts resulted in a 32% increase in cell number over control populations by 24 h. In contrast, the same amount of protein-free beads or beads with bovine serum albumin (BSA), ovalbumin, or SBTI attached did not significantly increase cell number over controls. This specificity was also observed over a wide range of bead concentrations.

Coverslip experiments

Our first attempts to determine if initiation by trypsin-beads resulted from trypsin released during incubation involved an approach where two populations of cells, one with and one without beads, shared the same medium. We cultured CEF on small glass coverslips and then added trypsin-beads (~ 225 beads per cell) to some of the cultures. Nearly all of the beads firmly adhered to the cells within 3 h. We then relocated coverslips with and without beads into new dishes. The beads remained tightly associated with the cells both during transfer of the coverslips and during the subsequent incubation. Regardless of the proportion of coverslips with and without

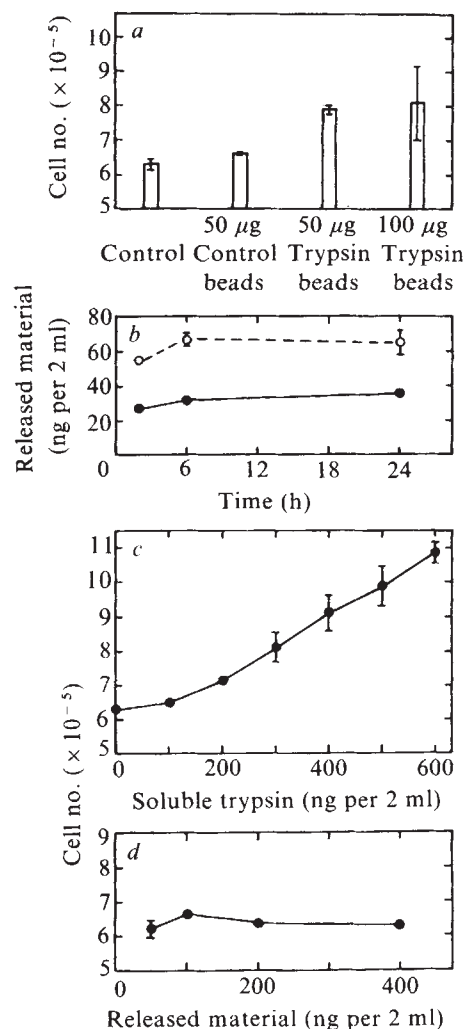


Fig. 2 Amount of material released from trypsin-beads during incubation with CEF and the mitogenic effect of different amounts of trypsin-beads, soluble trypsin, and released material. ^{125}I -trypsin (described in Fig. 1) was covalently attached to polystyrene beads as described in Table 1. $50\text{ }\mu\text{g}$ and $100\text{ }\mu\text{g}$ of the ^{125}I -trypsin-beads were added to quiescent CEF (Table 1). **a**, Cell number 24 h after bead addition. **b**, Amount of TCA precipitable material released into the medium from $50\text{ }\mu\text{g}$ (●) and $100\text{ }\mu\text{g}$ (○) of ^{125}I -trypsin-beads during the 24 h incubation. In the same experiment indicated amounts of soluble trypsin (**c**) and material released during the 2-h preincubation of ^{125}I -trypsin-beads (**d**) were added to cultures of quiescent CEF and the cell number counted 24 h later. The absence of an error bar means it was smaller than the symbol. Before using TCA-precipitable radioactivity as a measure of the accumulated mitogenic activity, it was necessary to determine if material released from the beads was degraded to TCA soluble material during incubation. $0.5\text{ }\mu\text{g}$ of soluble ^{125}I -trypsin added to CEF cultures remained TCA precipitable during a 24-h incubation. Therefore, it is unlikely that material was released in an active TCA-precipitable form and then degraded.

Table 1 Effect of bead preparation on CEF cell number

Addition	Cell number ($\times 10^{-5} \pm \text{s.d.}$)	% Increase over control
None	6.28 ± 0.30	
Beads alone	6.27 ± 0.02	-0.2
BSA-beads	6.48 ± 0.28	3.2
Ovalbumin-beads	6.56 ± 0.06	4.5
SBTI-beads	6.51 ± 0.24	3.7
Trypsin-beads	8.32 ± 0.16	32.5

Secondary chick embryo fibroblasts, obtained as described by Rein and Rubin³¹, were seeded at 5.5×10^5 cells per 35-mm dish in 2 ml of medium 199 containing 2.0% Tryptose phosphate broth and 2% chicken serum. After 4 h the medium was changed to medium containing 0.02% chicken serum. After 48 h, 50 μg of beads alone or beads with the indicated proteins attached were added to the quiescent cultures and cell number was determined 24 h later. The data in all tables and figures are means of duplicate determinations. Trypsin-beads were prepared as follows: 500 μg of trypsin (three-times recrystallised, Worthington) was mixed with about 5 mg of carboxylate-modified polystyrene beads (0.926 μm diameter, Dow Diagnostics) in 0.1-M sodium acetate, pH 6.0, and linked by addition of water soluble carbodiimide (1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide metho-*p*-toluene sulphonate, Sigma) to 0.1 M (ref. 32). After 4 h of stirring at 4 °C the beads were *a*, washed five times by centrifugation (10,000*g* for 10 min) through the sodium acetate buffer; *b*, pre-incubated at 37 °C for 2 h in conditioned medium (taken from CEF cultures 40 h after changing to 0.02% serum medium), and *c*, washed and resuspended in 2.5 ml of phosphate buffered saline (PBS). 50 μg of these beads (1.2×10^8 beads) had approximately 2.0 μg of trypsin attached. All other protein-beads were prepared as described for trypsin. During the above 2 h pre-incubation there was considerable release of trypsin-derived material. Therefore, we always included this pre-incubation to minimise the amount of material released when beads were added to CEF.

beads in any single dish, we found a 50% increase in cell number after 36 h on coverslips previously overlaid with trypsin-beads, but no increase in cell number on coverslips without them. Moreover, the amount of initiation observed in bead-contacted cells was not decreased by doubling the volume of the medium. Because initiation of CEF is very sensitive to changes in soluble trypsin concentration (see Fig. 2c), these experiments strongly suggested that released trypsin was not responsible for the initiation observed with trypsin-beads.

Measurement of released material and assessment of its activity

To quantitate the release of trypsin from beads during incubation, we attached ¹²⁵I-trypsin to polystyrene beads as described in Fig. 2. When these trypsin-beads were added to CEF, a small amount of material was released into the medium. Therefore, it was important to determine if the released material was intact trypsin and if it retained mitogenic activity. When fractionated on Sephadex G-75 columns, only a small portion of the released material was intact trypsin (23,000 molecular weight). The majority was peptide fragments with molecular weights of 12,000 or less. To determine if this material was active

necessitated a very sensitive measure of mitogenic activity. Fortunately, we observed that during autodigestion of ¹²⁵I-trypsin the loss of mitogenic activity (as well as proteolytic activity) closely followed the decrease in trichloroacetic acid (TCA)-precipitable radioactivity (Fig. 1). Thus, simply measuring the TCA-precipitable radioactivity released from ¹²⁵I-trypsin beads during incubation with CEF indicated the maximum amount of released mitogenic activity.

Figure 2 shows representative experiments which demonstrated quantitatively that the amount of material released from trypsin-beads could not account for the observed initiation of cell division. The addition of 50 μg of trypsin-beads to quiescent CEF cultures resulted in a 25% increase in cell number over controls by 24 h (Fig. 2a). During this 24 h incubation 35 ng of TCA precipitable material was released into the medium (Fig. 2b). It is clear that this 35 ng of released material could not account for the initiation, since addition of 100 ng of soluble trypsin had virtually no effect on cell number by 24 h (Fig. 2c). In fact, to cause a 25% increase in cell number would require approximately 280 ng of soluble trypsin (Fig. 2c). Thus, even if all of the released TCA-precipitable material were active, eight times more would be required to account for the initiation observed with 50 μg of trypsin-beads. Addition of 100 μg of trypsin-beads brought about no additional increase in cell number even though twice as much material was released into the medium (Fig. 2a, b). It is noteworthy that adding up to 400 ng of released material had no effect on cell number within 24 h (Fig. 2d). Thus, even at high concentrations released material was not able to initiate CEF.

Can trypsin be released from beads directly into CEF?

The above experiments ruled out the possibility that release of trypsin into the medium caused the observed initiation. Another possibility, however, is that active trypsin could be released directly into the cells without appearing in the medium. We attempted to examine this possibility by incubating CEF with ¹²⁵I-trypsin-beads, separating the beads and cells, and determining if any radioactivity remained with the cells. In these experiments, however, we were unable to separate most of the polystyrene beads from the surface of intact cells by treatments with trypsin, hyaluronidase, neuraminidase, collagenase, or low concentrations of detergents. Therefore, we rinsed the cells, dissolved them in sodium dodecyl sulphate (SDS), collected the beads on Millipore filters, and measured the amount of radioactivity on the filters and in the filtrate (Table 2). As shown, soluble trypsin passed through the filters while trypsin attached to the beads was retained. SDS treatment alone caused the release of an average of 2.6% of the bound radioactivity, but no additional soluble radioactive material was observed when the beads were incubated with the cells for periods up to 24 h. These experiments ruled out significant direct uptake of trypsin from the beads by CEF.

Table 2 Recovery of ¹²⁵I-Trypsin attached to beads

Preparation	Incubation conditions	Soluble radioactivity (c.p.m.)	Bead-attached radioactivity (c.p.m.)	% Recovered on beads
¹²⁵ I-Trypsin-beads (preparation A)	SDS treatment alone	1,035	26,017	96.2
	3 h with CEF	688 ± 61	$19,375 \pm 1,770$	96.6
	6 h with CEF	606 ± 13	$21,099 \pm 3,958$	97.2
	24 h with CEF	209 ± 4	$16,118 \pm 409$	98.7
¹²⁵ I-Trypsin-beads (preparation B)	SDS treatment alone	153 ± 16	$10,895 \pm 1,395$	98.6
	24 h with CEF	151 ± 17	$9,596 \pm 375$	98.5
Soluble ¹²⁵ I-trypsin	SDS treatment with CEF plus control beads	$12,731 \pm 2,023$	527 ± 55	4.0

¹²⁵I-Trypsin-beads prepared as described in Fig. 2 were added to quiescent CEF (see Table 1). After the stated incubation periods, medium and non-adherent beads were removed, the dishes were rinsed twice with PBS, and the monolayers were dissolved in 1 ml of PBS containing 2% SDS. The beads were then collected on Millipore filters (0.45 μm), the dishes and filters washed twice with PBS, and the filters and soluble material (including washes) counted. As controls, trypsin-beads from each preparation were added to dishes treated with the SDS solution and filtered. In addition, we added soluble ¹²⁵I-trypsin and ovalbumin-beads to CEF monolayers, immediately dissolved these with SDS, and filtered them. Since soluble trypsin was not retained on the filters in these conditions, it is unlikely that material released from beads during incubation became reassociated with beads or cellular material and was retained on the filters.

Visualisation of bead-cell interaction

When CEF are treated with soluble trypsin or thrombin they undergo morphological changes in addition to their increase in cell number. Such cells appear more elongated and slightly retracted with fewer cell-cell contacts and decreased cytoplasmic spreading¹⁶. Treatment with soluble trypsin also has been reported to cause the release of a protein of 250,000 molecular weight (the 'LETS' or '250K' protein) from the surface of these cells^{14-16, 27}. Figure 3*a* and *b* shows CEF 24 h after addition of 50 μ g of trypsin-beads and control phosphate buffered saline (PBS), respectively. Notably, the trypsin-bead-treated cells had altered morphologies resembling that reported for CEF treated with soluble trypsin. In addition, analysis of medium from cells treated with trypsin-beads revealed that a protein of approximately 250,000 molecular weight was released from the cells into the medium. These results indicated that trypsin-beads had a proteolytic effect on the surface of CEF similar to that of soluble trypsin.

Some of the trypsin-beads were endocytosed by CEF during incubation. Figure 3*c* and *d* shows trypsin-beads in contact with and inside of cells. Based on stereometric reconstruction from cross sections of 150 cells²⁸, we estimated that less than 4 of approximately 225 beads added per cell were endocytosed by 9 h, a time corresponding to the peak of DNA synthesis¹¹. It is important to emphasise that of 100 internalised beads observed in cross sections, all appeared to be surrounded by intact membranes (see Fig. 3*d*). Therefore, even if these few internalised trypsin beads were involved in initiation of cell division, they acted on the plasma membrane and did not interact directly with the cytoplasm. Furthermore, the process

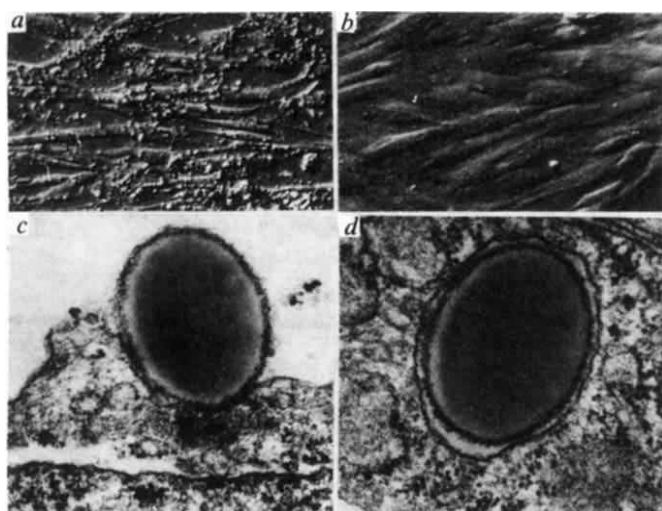


Fig. 3 Effect of trypsin-beads on CEF morphology and the ultrastructure of the bead-cell interaction. *a, b*, Photomicrographs of CEF cultures 24 h after addition of *a*, 50 μ g of trypsin-beads, and *b*, 40 μ g of PBS as a control (850 $\times$, Hoffman optics). *c, d*, Electron micrographs ($\times 70,000$) of CEF 6-h after addition of trypsin-beads. For electron microscopy, cell monolayers in 35-mm Falcon plastic dishes were fixed for 30 min at 4 $^{\circ}$ C with combined glutaraldehyde (1.25%) and osmium tetroxide (1%) in 0.1 M phosphate, pH 7.4 (ref. 34). They were then rinsed, stained with uranyl acetate, dehydrated in ethanol and embedded in Epon 812 (ref. 35). After polymerisation the Epon monolayers were heated to 85 $^{\circ}$ C and separated from the plastic dishes. Thin sections were cut perpendicular to the monolayer and were post stained with uranyl acetate and lead citrate. When beads were in contact with the surface of CEF we often observed thickened filamentous material beneath the plasma membrane (note Fig. 3*c*). But, because some beads were observed inside the cells (Fig. 3*d*) such thickenings may be involved in binding or endocytosis and not be related to initiation. That trypsin-beads were endocytosed by CEF was demonstrated by serial sectioning and by using tannic acid-glutaraldehyde as a primary fixative to show that the membranes surrounding the beads were not accessible from outside the cells³⁶.

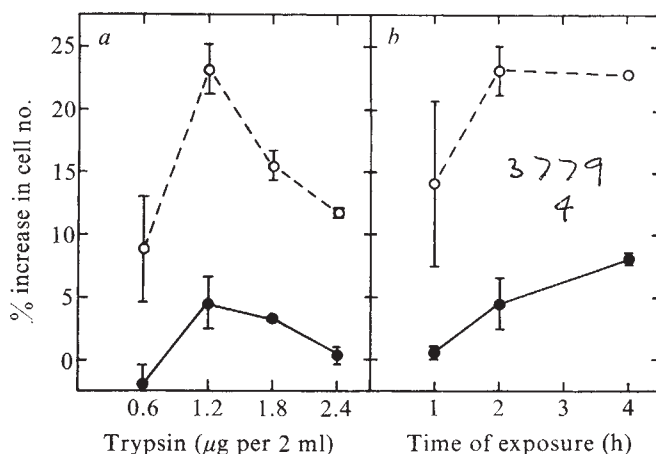


Fig. 4 Inhibition by SBTI-beads of cell division initiated by soluble trypsin. Indicated amounts of soluble trypsin were added to quiescent secondary CEF (as described in Table 1). After the indicated periods medium containing trypsin was removed and replaced with conditioned medium taken from unstimulated parallel cultures. 120 μ g of SBTI-beads were added to half of the cultures. Cell number was determined 24 h after the beginning of trypsin exposure. The absence of an error bar means it was smaller than the symbol. *a*, Effect of 2-h exposure to stated trypsin concentrations. *b*, Effect of stated exposure times to 1.2 μ g of soluble trypsin per 2 ml of medium. Conditioned medium alone (○); conditioned medium plus SBTI-beads (●). Experiments with ¹²⁵I-SBTI-beads demonstrated that ~95% of the SBTI remained attached to the beads during incubation with CEF with or without trypsin.

of bead endocytosis itself did not trigger cell division because ovalbumin-beads, which did not stimulate DNA synthesis or cell division, were also taken up by CEF.

Initiation by soluble trypsin can be inhibited at the cell surface

Assuming that soluble trypsin acts in the same manner as immobilised trypsin and initiates proliferation by action at the cell surface, then it should be possible to inhibit such initiation with a trypsin inhibitor bound to polystyrene beads. Indeed, in early experiments we found that addition of 120 μ g of SBTI-beads to CEF inhibited 95% of the cell division initiated by 0.5 μ g of soluble trypsin. But, measurements of soluble trypsin in the medium revealed that part of the inhibition could be attributed simply to a decrease in the concentration of soluble trypsin.

To bypass the problem of interpreting changes in the concentration of soluble trypsin, experiments were performed where only cell-associated trypsin was exposed to the SBTI-beads (Fig. 4). In these experiments we incubated CEF with trypsin for periods up to 4 h, and then removed the medium (containing trypsin) and replaced it with conditioned medium alone or conditioned medium plus SBTI-beads. Figure 4*a* shows the effect of 2-h incubations of CEF with various concentrations of trypsin. The highest level of initiation was a 23% increase over control cells with 1.2 μ g of added trypsin (0.6 μ g ml⁻¹). Addition of 120 μ g of SBTI-beads ($\approx$ 680 beads per cell) with fresh conditioned medium after the 2-h incubation with trypsin inhibited this stimulation by more than 80%. Even after a 4-h exposure to trypsin at this concentration, approximately two-thirds of the initiation could be inhibited by SBTI-beads (Fig. 4*b*). The same number of SBTI-beads only slightly inhibited serum stimulation of CEF. Thus, the inhibition was specific for initiation by soluble trypsin at the cell surface. Moreover, addition of ovalbumin beads did not inhibit initiation by soluble trypsin. These results do not exclude the possibility that soluble proteases which accumulate inside cells during long exposures might have a role in initiation of cell division. It is, however, difficult to explain how SBTI-beads

inhibited initiation after 4-h trypsin exposures if the primary site of trypsin action during this period were not at the cell surface.

Transmembrane action of trypsin and other growth factors

Several growth promoters have been reported to act at the cell surface. For example, there are reports that concanavalin A, phytohaemagglutinin, and pokeweed mitogen initiate DNA synthesis in lymphocytes while immobilised on Sepharose beads or plastic surfaces^{5,6}. There is, however, a report that preparations of these mitogens which are active when soluble lose all of their mitogenic activity when immobilised by techniques similar to those used in the earlier reports⁹. Thus, it is uncertain whether these mitogens act at the cell surface or not.

Two other peptide factors immobilised on Sepharose beads have also been reported to act at the cell surface. (1) There are reports that insulin-Sepharose is biologically active^{2,3}, but recent experiments have demonstrated that all or most of its biological activity can be attributed to released soluble insulin^{7,8}. (2) Nerve growth factor-Sepharose has been reported to stimulate neurite extension from sympathetic ganglia⁴. In these experiments, controls were included to measure release from the beads. These controls, however, were indirect and involved diffusion through plasma clots. With such controls it is not possible to exclude release from beads directly into the cells. In addition, the validity of experiments with Sepharose-immobilised peptide factors has been challenged on the basis that incubation of these preparations with proteins can cause release of 'superactive' factors (often with altered specificity)¹⁰. These problems with Sepharose preparations and the necessity for definitive controls led us to seek a more suitable method for immobilisation and to directly quantitate material released into the medium and into cells.

The experiments we report with trypsin attached to polystyrene beads demonstrate that trypsin action at the cell membrane is sufficient to initiate proliferation of CEF. In addition, the inhibitory effect of SBTI-beads on initiation by soluble trypsin indicates that soluble trypsin can also act at the cell surface to initiate proliferation. It remains to be seen how the

mechanism of initiation by trypsin relates to that of other growth factors. It has been suggested that proteases may bind to or cleave receptors for more specific growth factors, and thus mimic their actions^{29,30}. If so, the ability of trypsin to initiate CEF by action at the cell surface implies that these growth factors also act at the cell surface.

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letters to nature

The binary pulsar observed at four radio frequencies

PSR1913+16 is still the only known pulsar which is a member of a binary stellar system, as such, it offers the possibility of several new investigations into both the nature of pulsars and general relativity. Unfortunately, the pulsar is very weak and previous observations^{1,2} have only been made with the 1000 ft telescope at Arecibo at a frequency of 430 MHz. We report here observations at four radio frequencies which show that this pulsar has properties which are similar to those of the other pulsars.

The 250ft MK IA telescope at Jodrell Bank was used for the observations, in 1975 and 1976, at frequencies of 327, 408, 610 and 1,420 MHz. Dispersion-removing receivers were used at all frequencies providing total bandwidths of 1, 1, 4 and 4 MHz respectively, each of which was divided into 32 equal contiguous sub-bands. The time resolution is then limited either by dis-

persion within these sub-bands or by the integration bin size used. The integrations were carried out by forming on-line sub-integrations of 5 or 10 min in length, using the orbital ephemeris published by Taylor *et al.*². These sub-integrations were added together later for total periods of between 8 and 12 h, amounting to between 1 and 1½ orbital periods of the binary system. This was achieved using the data reduction technique described by Lyne *et al.*³, after slight modifications to compensate for the motion of the pulsar in the binary system.

The pulsar integrated profile obtained at each of the four frequencies is shown in Fig. 1 in which the whole of the 59 ms period of the pulsar is represented. The observations were made on different occasions, and have been arbitrarily aligned in phase. In all cases, the profile seems to have two main components whose separation does not vary significantly with frequency. The profile shape is characteristic of many other pulsars, although the profile covers a larger fraction of the pulsar period (~20%) than for the majority of pulsars. An

unusual feature is that there does not seem to be any significant broadening of the pulses at low frequencies by scattering in the interstellar medium. Any broadening present amounts to only a few ms at 327 MHz which is rather small for a pulsar having a dispersion measure of $167 \text{ cm}^{-1} \text{ pc}$ (ref. 4).

The spectrum of the pulsar derived from these observations is of some interest and is shown in Fig. 2. There is a certain amount of variability in intensity over timescales of months in some pulsars⁵, if such a variation is occurring in PSR1913+16 the measurements in the diagram, being spaced by some months, may have effective errors somewhat greater than the errors of measurement indicated. The spectral index in the range 327 MHz to 1420 MHz is measured as -1.5 ± 0.3 which is typical of many of the other pulsars⁶. Further observations, more closely spaced in time will be required to confirm this value.

Observations are continuing with the aim of examining the evolution of the orbital parameters of the binary system and of studying any associated changes in the intensity and polarisation of the radio emission. In particular, if precision of the pulsar occurs it may be possible to view the pulsar from a different rotational latitude, which is something that is not possible for any other known pulsar.

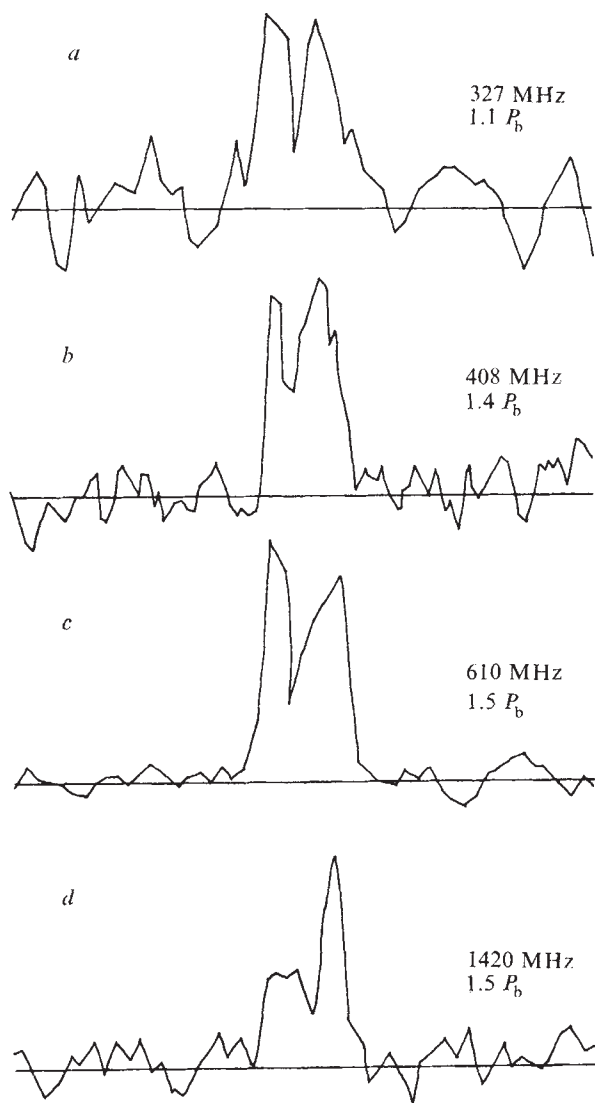


Fig. 1 The PSR1913+16 pulse profile at four radio frequencies. *a*, 327 MHz, February 1976; *b*, 408 MHz, May 1976; *c*, 610 MHz, April 1975; *d* 1420 MHz, June 1975. In each case, the whole of the 59-ms pulsar period is shown. The integration time in terms of the binary orbital period $P_b \approx 7.7 \text{ h}$ is also indicated.

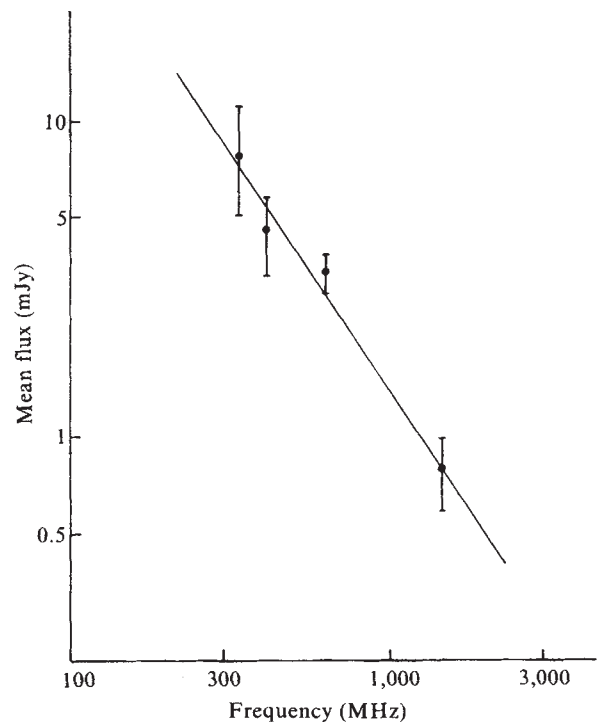


Fig. 2 The radio spectrum of PSR1913+16.

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X-ray bursts from dense clouds

X-RAY heating of matter accreting on to a compact object can create unstable flows leading to irregular X-ray emission¹⁻⁴. Previous discussions of this instability have concentrated on the case of spherically symmetrical infall onto a stationary object. The rise-time of X-ray variations is then related to the free-fall timescale from the choking or accretion radius, whichever is relevant. Phenomena due to the presence of a magnetosphere⁴ may, however, reduce this estimate. It is not clear that bursts with duty cycles as short as 10^{-3} can arise from the heating instability. Such bursts are observed, and here I investigate the effect of the motion of a compact object through a relatively dense medium. The passage of the compact object through a denser shell, which builds up outside the X-ray-heated gas, produces a short burst of X-rays. This creates yet another hot region leading to the repetition of bursts on a crossing time. Such behaviour may be relevant to the weak bursts possibly associated with the Cometary Globule NGC 5367 (ref. 5), and the much shorter timescale luminous X-ray bursts⁶, some of which are associated with globular clusters.

Consider first what happens when a neutron star or white dwarf, with velocity v km s⁻¹, accretes from a dense cloud such as NGC 5367. Much of the material is probably in the form of molecular hydrogen of density $n_4 \sim 1$ where $n \sim 10^4$ n₄ cm⁻³ at a temperature $T_2 < 1$ where $T = 10^2 T_2$ K (ref. 7). X rays with energies $\epsilon \gtrsim 2$ keV pass through such a cloud with little attenuation since it is only a few parsecs in size. The accretion radius

$$R_A \simeq \frac{10^{16}}{(T_2 + v^2)} \text{ cm}$$

yields luminosities $L \sim 10^{39} n_4 (T_2 + v^2)^{-3/2} \text{ erg s}^{-1}$ in the case of a neutron star, and $L \sim 5 \times 10^{37} n_4 (T_2 + v^2)^{-3/2} \text{ erg s}^{-1}$ in the case of a $1 M_\odot$ white dwarf. The short-wavelength radiation, $\epsilon > 13.6$ eV from such luminous sources would heat the accretion flow above 10^4 K, resulting in instabilities on a free-fall timescale, $t_{\text{ff}} \sim 10^{11} R_{16}^{3/2}$ s, where R_{16} is the choking radius in units of 10^{16} cm.

Short timescales occur if the compact object has a substantial velocity, $v \gtrsim 100$ km s⁻¹. Many lone neutron stars may perhaps be expected to travel at such velocities, and I now concentrate on this possibility. An accretion radius $R_A \simeq 10^{11} v_{300}^{-2}$ cm yields a luminosity $L \sim 3 \times 10^{31} n_4 v_{300}^{-3} \text{ erg s}^{-1}$ and $t_{\text{ff}} \sim 3 \times 10^3 R_{11}^{3/2}$ s. The precise form of the X-ray spectrum is unknown, but the blackbody temperature of low-luminosity neutron stars is $\sim 10^5$ – 10^6 K, and thus much of the radiation may emerge at energies $\epsilon < 1$ keV. The absorption of a number, N_{ph} , of soft X rays ionises the surrounding region out to a radius R_i such that $N_{\text{ph}} \simeq \frac{4}{3} \pi R_i^3 n$, provided that the optical depth through R_i , $n \sigma R_i$, exceeds unity. The optical depth criterion demands that $R_i \gtrsim 2 \times 10^{13} n_4^{-1}$ cm, given⁸ the photoelectric absorption cross section, σ . This in turn requires $N_{\text{ph}} \gtrsim 4 \times 10^{44} n_4^{-2}$, which is adequately supplied by $\sim 10^{35}$ erg of ~ 50 eV photons. The upper limit on the timescale over which the photons can be supplied is a crossing time of R_i , $t_{\text{cr}} \sim 10^6$ – 10^7 s, the recombination timescale being much larger, $t_r \sim 10^9 n_4^{-1}$ s. An ionised region can therefore form if a few per cent or more of the neutron star luminosity emerges in the 13.6–50-eV band, assuming $v_{300} \sim 1$.

The absorbed energy not only goes into ionisation and dissociation of hydrogen molecules and atoms, but also into heating. The resultant temperature, $T \sim 10^4$ – 10^5 K will cause a large pressure difference to exist between the ionised region and the surrounding molecular cloud. R_i thus expands supersonically at $v_s \sim 30$ km s⁻¹ into the cool exterior, shock heating and dissociating the hydrogen molecules. The expansion is not adiabatic due to the loss of energy in dissociation. The temperature drops by a factor of ~ 10 to $\sim 3,000$ K in this process⁹, resulting in an overall 40-fold increase in density behind the shock. The material then cools to ~ 100 K in $\sim 10^5$ s provided that the ionised fraction in the cooling gas exceeds 10% (ref. 10). The overall density enhancement in what is then essentially an isothermal shock may thus amount to a factor of $\sim 10^3$, residing in a shell of thickness $d \sim 10^{-3} \Delta R_i$, where ΔR_i is the amount by which R_i has increased. The ionised zone approximately resembles a stand-off cone lying with its axis along the direction of motion of the neutron star.

Assume that a transient increase in luminosity, perhaps due to a density enhancement, causes a larger, roughly spherical, ionised region to form. Significant deceleration of R_i will only occur when an equal mass has been swept up in the shell, that is $\Delta R_i/R_i \sim 0.3$. When the compact object reaches the shell, $\Delta R_i/R_i \sim v_s/v_{300} \sim 0.1$, and thus $d \sim 10^{-4} R_i$. The density enhancement in the shell causes L to rise to $\sim 3 \times 10^{34} v_{300}^{-3} \text{ erg s}^{-1}$ on t_{ff} , subsequently dropping on a similar timescale after d has been crossed. The total burst energy is $\sim 10^{38}$ – 10^{39} erg, only a few per cent of which need be in photons creating a further ionised region beyond the shell. The X-ray bursts then repeat on intervals of $t_{\text{cr}} \sim 10^7$ s. The relatively short cooling time of the dense shell prevents it from being evaporated by the low steady X-ray flux.

The observed bursts, which may have come from NGC 5367, were separated by ~ 200 d and each lasted for several hours⁵. The observed 2–10-keV luminosity was $\sim 10^{34} \text{ erg s}^{-1}$ during each burst. The above discussion suggests that a lone, fast moving,

neutron star may be responsible. The probability that such a neutron star intersects a globule of volume $\sim 10 \text{ pc}^3$ may be as high as 0.03, if there are as many as 10^9 suitable neutron stars within a scale height of ~ 500 pc about the galactic disk. The discussion is perhaps more relevant in indicating the possibilities for detecting burst like phenomena from molecular clouds optically thin to X rays within the plane of the Galaxy. The high galactic latitude ($b \sim 20^\circ$) of the NGC 5367 events probably aided in their detection. Slower moving neutron stars, $v \sim 50$ km s⁻¹, may lead to events of longer duration and higher luminosities, possibly resembling Aquila X-1 and 3U1630–47 (refs 11, 12). White dwarfs may also give rise to weaker burst phenomena, as approximately half the accretion luminosity emerges in the ultraviolet¹³, thereby increasing their potential to create ionised zones.

A slow moving neutron star, $v \sim 10$ km s⁻¹, moving through a region of density $n \sim 10^8 \text{ cm}^{-3}$ can give rise to very high luminosities, $L \gtrsim 10^{38} \text{ erg s}^{-1}$. Compton heating of the accreting gas, as well as radiation pressure effects, may choke the flow at relatively small radii, $R_c \sim 10^{11} \text{ cm} \ll R_A$ (ref. 4). Accretion may still proceed beyond R_c , and a dense, convectively unstable shell may result. Luminous X-ray bursts repeating on a timescale $t_{\text{cr}} \sim 10^4$ – 10^5 s, caused by the passage of the neutron star through the dense shell enveloping the hot bubble produced from the previous burst, can occur if $t_{\text{cr}} < t_s$, the timescale on which the shell dissipates. The actual choking process is, as discussed in ref. 4, likely to be very complex and no detailed estimate is attempted here. Magnetospheric effects, for example, can modify the structure of the burst. Nevertheless, such bursts may in principle occur, and a suitably dense region may occur in the outer envelope, $R \sim 10^{14}$ cm of a red giant star undergoing mass loss. An accretion disk may form if the accreting material has sufficient angular momentum, as might be the case if the neutron star orbits the giant. The presence of a disk would stop bursts from occurring, but, as it depends on R_A^4 , however, a disk need not occur. Bursts such as those observed from NGC 6624 (3U1820–30) (ref. 14) may be related to this process. Optical photographs^{15,16} of globular cluster cores indicate the presence of luminous red giants.

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Continuous increase of Hubble modulus behind clusters of galaxies

VARIOUS authors have suggested the existence of real fluctuations to Hubble's law (proportionality of redshift to distance) as a function of the position of sources on the sky map. Karoiji and Nottale¹ have examined the possibility of a variation of the Hubble constant H associated with the passage of incoming light through rich clusters of galaxies. We confirm here their positive answer by a more detailed and precise statistical

analysis. We show that there is an effective continuous effect depending on the relative distance of the line of sight of any given source to the centre of the intervening clusters. We thus conclude that we are dealing with a real physical effect, tied to the presence of these clusters.

It has been shown¹ that the Hubble modulus

$$h_m = \log V - 0.2 m = \log H - 0.2 M - 5$$

where V is radial velocity, H the Hubble constant, m and M , apparent and absolute magnitude of a galaxy, is significantly higher for galaxies located within and behind rich clusters of galaxies (denoted region B) than for intercluster objects (denoted region A). This result has been suggested on four independent samples¹⁻⁴ and confirmed by analysis of brightest cluster galaxies⁵. The method used previously—comparison of the mean values $\langle h_m \rangle_B$ against $\langle h_m \rangle_A$ through an application of Student's t test, was a rather crude one. We present here a new method of analysis of h_m variations as a function of a continuous variable. Of course, if each independent sample had been rich enough, it would have been advisable to construct h_m 'isovalues' on the sky map. Since this is not the case, we have devised an alternative method.

First, rich clusters of galaxies being defined as in ref. 1, we classify galaxies as:

(1) Cluster galaxies, if they are situated within the limits (as defined by Zwicky *et al.* in CGCG⁶) of a cluster, and if their radial velocities V are within an interval of 6σ around the mean cluster velocity (the mean value of σ for the clusters used in this analysis is around 750 km s^{-1}), that is

$$V_{cl} - 3\sigma(V) \leq V \leq V_{cl} + 3\sigma(V)$$

(2) Galaxies more distant than a cluster if $V > V_{cl}$ outside Zwicky's limits or $V > V_{cl} + 3\sigma(V)$ inside them.

Second, we refer each galaxy to a subsample of clusters nearer than it, or to which it belongs, and calculate the ratios θ/R , where θ represents its angular distance to a cluster centre, and R the angular cluster radius. Then we define a parameter α as the minimum of θ/R values, that is, $\alpha = \min \theta/R$. Centres and radii of clusters are taken from ref. 6.

Finally, h_m values are plotted with respect to α for a given sample of galaxies.

We present here the results obtained on the sample of Markarian galaxies identified in ref. 6, described and listed in ref. 4 (apparent magnitudes taken from ref. 6). This sample is of course magnitude limited ($m \leq 15.7$), which implies the presence of a Malmquist bias (distant galaxies are brighter in the mean than nearer ones) so that the h_m values increase with radial velocity. So we have built an unbiased subsample by taking only the brightest Markarian galaxies, $h_m > 0.96$. The clusters of galaxies used here are listed in ref. 3.

Figure 1 is a plot of h_m against α (for $\alpha \leq 6$). There is clearly a continuous decrease of h_m for $0 \leq \alpha \lesssim 3$. A quantitative analysis gives highly significant results.

First we have studied the distribution of h_m values subdivided with respect to α into bins of 10 points each (Table 1). If cluster galaxies (4 points) are excluded, the first bin yields $\langle h_m \rangle = 1.170 \pm 0.031$ ($0.4 \leq \alpha \leq 1.7$) instead of 1.144 ± 0.029 . These values might be compared with h_m for $\alpha \gg 1$, which are of the order of 1.05 ± 0.02 (Table 1).

A second method is to find the regression curve corresponding to h_m against α values. It is clear from Fig. 1 that a single line cannot account for the whole distribution. There is a strong decrease up to $\alpha \approx 3$, and for $\alpha > 3$, the distribution is practically flat. Indeed, the equation of the least-square line for $\alpha \leq 3.3$ (cluster galaxies excluded) is $h_m = 1.217 - (0.0567 \pm 0.016)\alpha$, the correlation coefficient being 0.48, which means

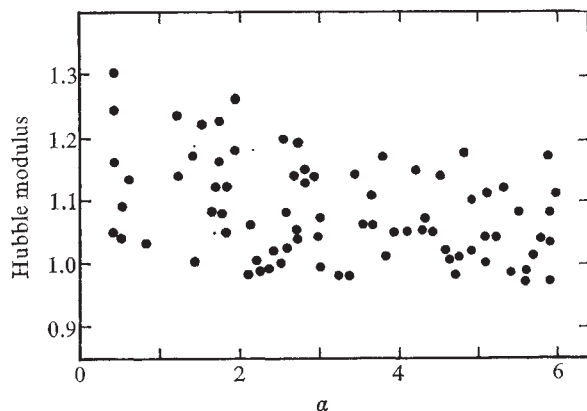


Fig. 1 Hubble modulus against normalised projected distance to nearer cluster $\alpha = \theta/R$ for unbiased sample of Markarian galaxies ($h_m > 0.96$).

that the probability that no correlation exists between h_m and α is only 0.001. The results of the least-square linear fit for $\alpha \leq 3$ and $\alpha > 3$ are

$$\alpha \leq 3, n = 38, h_m = 1.207 - (0.0502 \pm 0.0183)\alpha, \rho = 0.41$$

$$\alpha > 3, n = 43, h_m = 1.080 - (0.0048 \pm 0.0109)\alpha, \rho = 0.07$$

There is practically no correlation when $\alpha > 3$. We think that a good fit of the distribution of Fig. 1 for galaxies behind clusters is then achieved with

$$h_m = 1.21 - (0.055 \pm 0.018)\alpha \text{ for } \alpha \lesssim 3, \text{ and } h_m = 1.05 \text{ for } \alpha > 3$$

As the preceding method gives the statistical significance of only part of the distribution we have also tried to give a numerical account of it by a unique curve: Fig. 1 suggests a law of the shape $\exp(-\alpha^2)$. We find the following best fit: $h_m = 1.051 + (0.099 \pm 0.025) \exp(-0.17\alpha^2)$, significant at the 4σ level (Fig. 2).

Finally, these results are confirmed by the analysis of the effect through the method of Karoji and Nottale¹. The mean value of h_m in region B (that is, $\alpha \leq 2$) is 1.141 ± 0.018 ($\pm$ s.e.m.), in region B* ($\alpha \leq 2$, cluster galaxies excluded) 1.153 ± 0.010 and in region A, 1.057 ± 0.008 . Then the differences are respectively 0.084 ± 0.017 and 0.096 ± 0.018 , significant at the 4.9σ and 5.3σ levels.

It is not the purpose of this letter to discuss possible observational explanations of this effect. This has been started in the original paper of Karoji and Nottale¹ and in refs 3 and 4. A complete analysis is now in preparation. We will only discuss two particular points.

It is possible that our method of classifying galaxies with respect to clusters has an influence on the effect. Indeed a bias

Table 1 Analysis of data from Fig. 1

(1)	α (2)	$\langle h_m \rangle$ (3)
1—10	0.4—1.2	1.144 ± 0.029
11—20	1.4—1.8	1.123 ± 0.023
21—30	1.9—2.5	1.068 ± 0.033
31—40	2.6—3.0	1.101 ± 0.018
41—50	3.0—3.8	1.056 ± 0.022
51—60	3.9—4.7	1.061 ± 0.016
61—70	4.7—5.4	1.057 ± 0.021
71—80	5.5—6.0	1.045 ± 0.021
81—85	6.1—6.8	1.056 ± 0.046

Data from Fig. 1 was analysed into bins of 10 points each. (1), Ranks of points in a bin; (2) α interval; (3), mean h_m in each bin $\pm$ s.e.m. $\sigma/\sqrt{n}$.

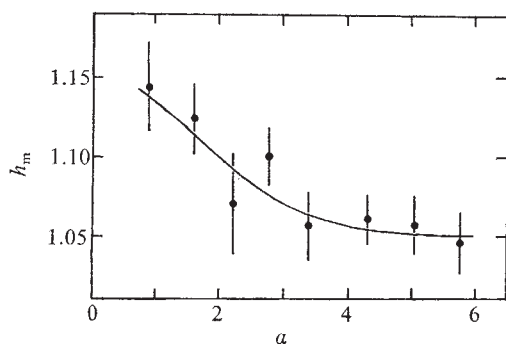


Fig. 2 Mean values of h_m against α in bins with errors bars and regression curve (see Table 1 and text). The last bin, containing only five points, is excluded.

could be present if clusters are actually greater than given by CGCG limits. So we have used another distance criterium, that is, galaxies are more distant than a cluster if $V > V_{cl} - 3\sigma(V)$. This change introduces no difference in our results, since only four galaxies are concerned by it, three slightly, and one ($h_m = 1.04$) having $\alpha = 5.1$ changed in $\alpha = 2.2$.

The second point is the possible relation with the Rubin *et al.* effect^{7,8} which is now interpreted by them as a motion of the local group with respect to ScI galaxies ($3,500 < V < 6,500$). The simplest way to take it into account is to correct our data for this motion ($V_{GL} = 450 \text{ km s}^{-1}$ towards $\alpha = 4 \text{ h } 12 \text{ min}$, $\delta = 36^\circ$). Our results are practically not affected by this correction. We obtain $\langle h_m \rangle_B - \langle h_m \rangle_A = 0.090 \pm 0.018$ ($t = 5.0$) and $\langle h_m \rangle_B - \langle h_m \rangle_A = 0.076 \pm 0.017$ ($t = 4.5$). It thus seems clear that if this solar motion exists, it is a small effect and independent of the Karoji-Nottale effect at the distances involved with our sample of Markarian galaxies ($V \sim 10,000 \text{ km s}^{-1}$).

Remembering that this analysis was performed on a subsample not affected by a Malmquist bias, our conclusion is that these results strongly support the existence of a dependence of redshift on the position of galaxies relative to concentration of matter, that is, clusters of galaxies.

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Polysaccharides and infrared spectra of galactic sources

OBSERVATIONS over the infrared waveband 2–30 μm available for a number of astronomical objects are shown here to be reconcilable with the transmittance properties of polysaccharides. Using an experimentally determined transmittance spectrum for cellulose we can readily relate astronomical data in the 2–4- μm , 8–13- μm and 15–30- μm wavebands and we obtain close fits to astronomical spectra in these several bands. From this detailed spectral agreement we consider it reasonable to infer the detection of interstellar polysaccharides. The identi-

fication of this highly complex macromolecule, presumably formed by an abiogenic processing of interstellar formaldehyde, could have a profound bearing on interstellar chemistry including the evolution of prebiotic molecules.

Physical conditions within dense molecular clouds are known to favour the production of organic molecules, probably through electron-exchange reaction chains initiated by the cosmic-ray ionisation of hydrogen and helium atoms^{1,2}. The majority of such molecules are fragile, however and are easily broken up by exposure to an appreciable intensity of starlight, which they will be if the parent clouds become dispersed to the normal density of the interstellar gas. Thus, since the interstellar gas undergoes alternating phases of compression and evaporation, there must be corresponding alternations of chemical complexity—molecular dissociations taking place during evaporation and association during compression. As Sagan has pointed out³ such alternations provide a selective process for the emergence of those chemical forms that can best withstand the adverse conditions of the evaporative phases.

With the exception of H_2 , H_2O and CO the most ubiquitous and very likely the most abundant molecule in the interstellar medium is H_2CO , probably formed by electron-exchange reactions^{1,2}. Formaldehyde would seem an obvious example of a fragile molecule forming within dense clouds which dissociates as soon as the clouds evaporate. This will certainly be the case for individual molecules, but formaldehyde has the remarkable property of polymerising in many ways, the polymers being far more resistant to break-up than the individual molecules.

The most refractory polymers are probably the polysaccharides, which have substructures built from H_2CO units, substructures with the empirical formula $(\text{H}_2\text{CO})_n$, where $n \geq 3$. The commonest polysaccharides, cellulose and starch with $n = 6$, are particularly stable because each $(\text{H}_2\text{CO})_6$ is able to form itself into a very stable ring, with the polysaccharide then becoming a chain of hexagonal ring substructures. Cellulose can maintain its structure (in a vacuum or in an inert atmosphere of low density) probably up to a temperature of around 625–900 K. (Laboratory data for wood cellulose indicate stability up to about 620 K (ref. 4), but in low-pressure interstellar conditions and in the absence of free oxygen there could exist polysaccharides similar to cellulose which can withstand temperatures up to about 900 K.)

In accordance with the selection occasioned by the alternating phases of compression and evaporation of the interstellar gas, can we expect fragile molecules of interstellar formaldehyde to have evolved into stable polysaccharides like cellulose and starch? Without supporting evidence, this would seem a bold conclusion, to say the least. Yet with the evidence presented here an affirmative answer does indeed seem warranted.

Interstellar solid material is strongly absorbing in two infrared bands, centred at 3 μm and at 10 μm . Because crystals of water-ice absorb at about 3 μm and magnesium silicate at 10 μm , it has seemed natural to attribute the infrared properties of interstellar 'dust' to a mixture of water-ice particles⁵ and magnesium-silicate grains^{6,7}. Indeed both these assignments are likely to be appropriate in certain types of localised region. But the detailed correspondence of a silicate-ice model to the observed infrared spectra of a wide range of galactic sources is not good, especially if one takes account of experimentally measured absorption spectra for silicate particles⁸. The failure to obtain detailed correspondences is the more disturbing because ice and silicate grains are rather simple materials, without much scope for their properties to be changed by other substances. For such simple materials the issue should be clear-cut, yet it is not. Rather it is frequently necessary to appeal to uncertainties in the astronomical models to explain away discrepancies between observation and theory.

This was the background which made it seem worthwhile to consider whether the infrared properties of interstellar 'dust'

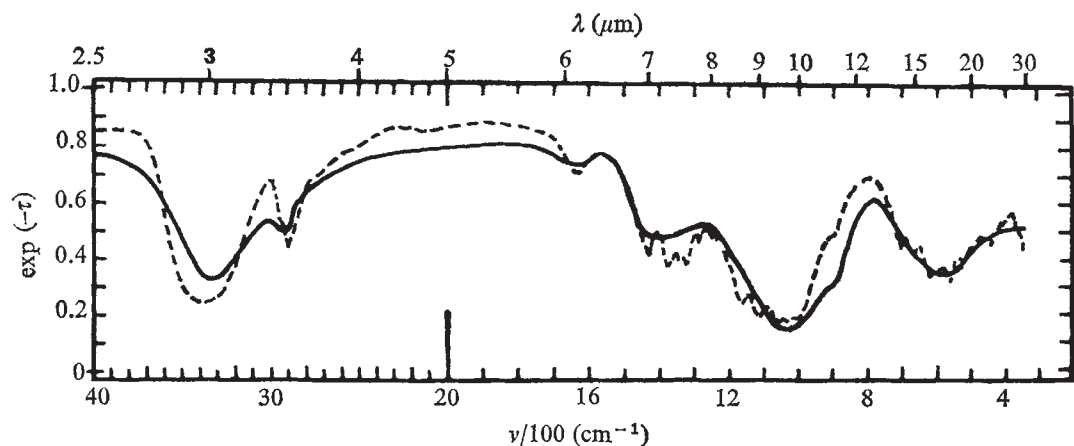


Fig. 1 Dashed curve is the transmittance data for cotton cellulose ('A cellulose 1') from ref. 9. Solid curve represents a smoother spectrum which we have estimated for an ensemble of related polysaccharides.

could be attributed to polysaccharides. Of all the polysaccharides, cellulose seems, because of its economic importance, to have been the most extensively studied, and the broken curve of Fig. 1 is reproduced from a calibrated study of the infrared spectra of cotton and its modifications⁹. Cellulose is immediately seen to have broad absorptions both at 3 μm and 10 μm , with the latter quite devoid of any unwanted spike of high transmittance, such as has been reported for silicates⁸. Transmittance values, $e^{-\tau}$, where τ is the opacity of the experimental sample, can be read off from the curve. Other polysaccharides possess similar transmittance properties, with slight wavelength displacements for the same general features (see ref. 12). The effect of such displacements for an ensemble of polysaccharides would therefore leave the general features unchanged, but must produce smoothing, as we have indicated by the solid curve of Fig. 1.

If the interstellar 'dust' has transmittance values corresponding to cotton cellulose, then the emission per unit wavelength band from an optically thin cloud of 'dust' at temperature T will be proportional to $\tau B_{\lambda}(T)$, where B_{λ} is the Planck function (This assumes the validity of the Rayleigh approxima-

tion for small particles with non-resonant absorptions. Technical issues relating to this assumption will be discussed elsewhere). Choosing the constant of proportionality to give a normalisation of 2.00 at $\lambda = 10 \mu\text{m}$, and taking $T = 175 \text{ K}$, we obtain the dashed curve shown in Fig. 2. The solid curve is calculated from the estimated smoother spectrum for a polysaccharide ensemble (solid line in Fig. 1). Since these calculated curves give excellent fits to the observations for the Trapezium Nebula^{10,11} (which are also given in Fig. 2) we consider that a

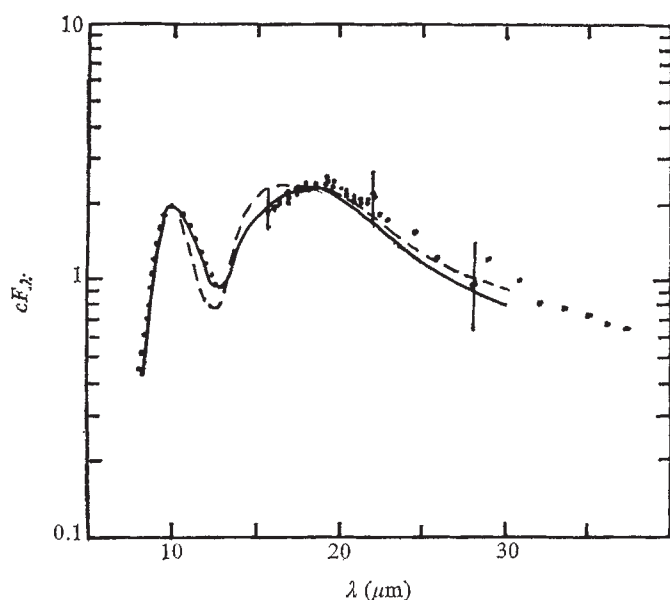


Fig. 2 Normalised flux from the Trapezium Nebula (refs 11, 12) (points) compared with emission from polysaccharide grains of temperature 175 K. Dashed curve is for cellulose (compare Fig. 1); solid curve corresponds to the polysaccharide ensemble spectrum as in Fig. 1.

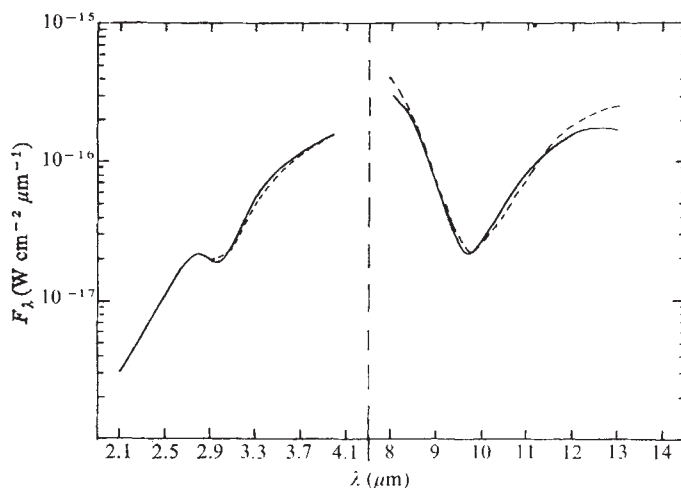


Fig. 3 Solid curve is a plot of the normalised flux against wavelength for an optically thin polysaccharide model with $T=43 \text{ K}$, $\alpha = 2.6$. Dashed curve is the observational data for the astronomical source $\text{H}_2\text{O} 610+18$.

prima facie case for the existence of interstellar polysaccharides has been established.

It has become well known among astronomers that material with the same emissivity as that which gives rise to the Trapezium spectrum also provides a thoroughly satisfactory explanation of the observed characteristics longwards of 8 μm for a wide range of other sources (see refs 13, 14). Hence the agreement of the cellulose data (and of the smooth curve of Fig. 1 in particular) with the Trapezium Nebula already ensures that the polysaccharide data must fit the observed characteristics longwards of 8 μm for the same wide range of sources. Our concern here will therefore be directed to the shorter-wave band 2–4 μm , since it is here that a more interesting situation arises.

We ask two questions. First, is there a satisfactory agreement of the polysaccharide data to observation for the shorter

wavelength band, 2–4 μm ? And second, can the shorter wavelength band be related in its intensity to the intensity of the longer wave band?

Taking the latter question first, Fig. 3 shows the relation of observations (broken curve) for the source H_2O 610+18 to the calculated curve (solid) given by the following model:

An optically-thin emitting cloud of temperature T lies behind a foreground cloud that is cool enough for its emission to be not relevant over the wavelength range under consideration here. The relative intensity is determined in the same way as for the Trapezium Nebula, except that an extra factor $\exp(-\alpha\tau)$ must be included to take account of absorption by the cool cloud. Thus the intensity is determined by $\tau B_\lambda(T) \exp(-\alpha\tau)$. The particular values chosen to represent the source H_2O 610+18 were $T = 430$ K, $\alpha = 2.6$. The answer to the first of the above questions is therefore affirmative, since the two wavebands are properly related (see also ref. 15, which gives a similar calculation for the source OH 26.5+0.6 taken over the very wide wavelength range of 2–30 μm).

The answer to the second question is also affirmative, as can be seen from the excellent fit of observation and calculation for the four sources given in Fig. 4. The model used here for the 2–4- μm waveband was the same as that described above, except that the hot emitting cloud was taken to be optically-thick. This has the effect of simplifying the formula used in the calculation, through the omission of the factor τ , to $B_\lambda(T) \exp(-\alpha\tau)$. The values chosen for T and α are shown in the

legend to Fig. 4. The details of these calculations, together with a discussion of several other sources, will be given elsewhere. In particular, the matching of the shorter and longer wavebands for these sources which involves a new feature in the model, will be discussed separately.

We conclude by noting that near 3 μm the sources of Fig. 4 show dips of very different depths one to another. Yet the same transmittance data was used in all cases—the difference arises from the choices of T and α . In the past, the dips at 3 μm have been attributed to water-ice, with *ad hoc* assumptions of different degrees of condensation of the ice necessary in order to explain the variability of the dips. There are no such *ad hoc* assumptions here. Nor is water-ice required at all.

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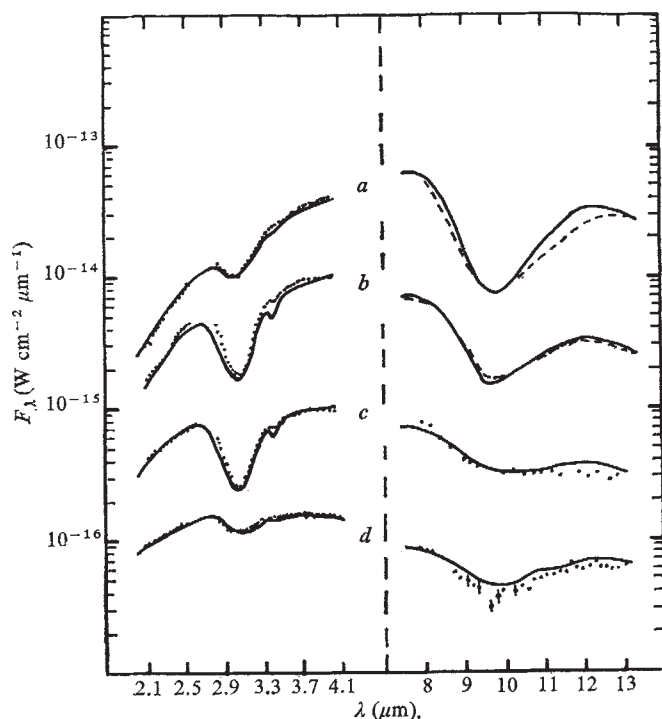
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Fig. 4 Cases *a–d* represent observational data (points) and normalised theoretical fluxes (solid curves). *a*, Observational points for CRL2591 $\times$ 20; theoretical curve on short wave side corresponds to an optically thick polysaccharide model with $T = 650$ K, $\alpha = 0.6$. *b*, Observational points for BN $\times$ 4; theoretical curve on short wave side is for an optically thick model with $T = 650$ K, $\alpha = 1.75$. *c*, Observational points for NGC2264IR; theoretical curve on short wave side is for optically thick model with $T = 800$ K, $\alpha = 1.7$. *d*, Observational points for CRL490 $\div$ 3; theoretical curve on short wave side is for optically thick model with $T = 850$ K, $\alpha = 0.25$. All the observational data are from ref. 12 and the theoretical curves are normalised to match the observations at one wavelength. The longwave calculations involve model refinements to be discussed elsewhere.



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Mechanism for formaldehyde polymer formation in interstellar space

CONSIDERING the properties of galactic dust clouds in the wavelength region 8–12 μm Wickramasinghe¹ concluded that formaldehyde undergoes polymerisation in interstellar space to polyformaldehyde. Applying more stringent constraints for matching infrared spectra of galactic sources over the waveband 2.5–30 μm Hoyle and Wickramasinghe^{2,3} recently argued for the identification of interstellar polysaccharides—which could be regarded as a further chemical evolution of formaldehyde polymers. A considerable fraction of all interstellar O and C may be locked up as some form of polymerised formaldehyde, making up the main component of interstellar dust. I argue here that molecular tunnelling in condensed formaldehyde is the only viable mechanism for the formation of formaldehyde polymers in interstellar conditions. Such a polymerisation process may be initiated by the action of ionising radiation.

The concentration of gaseous formaldehyde in dense molecular clouds with density $n_{\text{H}_2} \sim 10^4 \text{ cm}^{-3}$ is $n_{\text{H}_2\text{CO}} \sim 10^{-5} \text{ } 10^{-6} \text{ cm}^{-3}$. Using appropriate values for the heat of polymerisation of gaseous formaldehyde with the formation of crystalline polyoxymethylene (POM) ($\Delta H = 16,700 \text{ cal mol}^{-1}$ at 1 atm) and of the corresponding decrease in the entropy ($\Delta S = 42.6 \text{ cal mol}^{-1} \text{ deg}^{-1}$) (ref. 4) one can easily see that at the above-mentioned $n_{\text{H}_2\text{CO}}$ values interstellar POM would be thermodynamically stable below ~ 100 K. Furthermore, silicate grains of dimension $\lesssim 0.1 \mu\text{m}$ (ejected from cool stars) which many serve as the sites of condensation and polymerisation of formaldehyde have temperatures below ~ 20 K. Thus thermodynamical considerations confirm the possibility of the formation of stable polyformaldehyde

(and related polymers) in cold regions of interstellar space. In such circumstances the kinetics of polymerisation must have a decisive role.

Although direct polymerisation on the surfaces of interstellar mineral grains can be initiated by ionising radiation, the rate of propagation of polymer chains at very low temperatures could be vanishingly small⁵. Indeed, an Arrhenius-type extrapolation of our data⁶⁻¹⁰ on the kinetics of formaldehyde polymerisation at temperatures ~ 77 – 140 K to lower temperatures leads to the conclusion that the time for the addition of each new link to the growing polymer chain (with the activation energy $E \sim 2,000$ – $2,500$ cal mol⁻¹) would be $\tau_E \sim 10^4$ yr at 20 K, $\tau_E \sim 10^{30}$ yr at 10 K and $\tau_E \sim 10^{100}$ yr at 4 K. Nevertheless, the length of the chains (ν) of formaldehyde polymerisation which is close to the radiation-chemical yield of this process $G = g\nu$ (g is the number of primary active centres of polymerisation produced by 100 eV of absorbed X or γ radiation; $g \sim 1$) is quite large and reaches $\nu \sim 10^7$ at 140 K, $\nu \sim 10^5$ at 77 K and $\nu \sim 10^3$ at 4 K (refs 6–10). At first sight the value of $\tau_E \sim 10^4$ yr would seem compatible with the possibility of an Arrhenius-type, over-barrier growth of sufficiently long POM chains in interstellar grains, because the lifetime of dust clouds limited by their gravitational collapse or by cloud–cloud collisions is typically $\sim 10^5$ – 10^7 yr (refs 2, 3, 11). As will be shown below, however, an insurmountable obstacle for the over-barrier growth of polymer chains is the saturation of the radiation-chemical formation of chain propagating active centres—radicals or ions.

The steady-state concentration of radicals R_s achieved in conditions of external irradiation of molecular solids, even at very low temperatures ($\lesssim 4$ K), corresponds to rather small saturation values: $R_s \sim 10^{-3}$ – 10^{-2} and does not depend on the intensity of radiation. In principle there can be two conceivable causes of such saturation:

(1) Radicals and other active centres can be formed in solids only in the vicinity of various traps of energy—for example, defects and impurities. In such a case active centres formed by radiation can remain for a very long time (practically an infinite time) localised at some imperfections. Thus R_s is simply the fraction of pre-existing imperfections in the solid and obviously does not depend on the intensity of external radiation as long as the formation of additional defects by radiation itself can be neglected.

(2) The disappearance of radicals formed by radiation is due to recombination, and moreover the rates of both formation and recombination of radicals are proportional to the intensity of radiation¹³. In such circumstances R_s would again be independent of radiation intensity.

In this latter case² active centres are spatially delocalised and therefore R_s is equal to the part of the time (x_{act}) spent by each molecule in an active state. As shown by numerous experiments (see, for example, ref. 11) it is precisely this mechanism which causes the saturation of the yield of radicals at 77 K. Investigations¹³ of the formation and disappearance of radical pairs at much lower temperatures (down to 1.5 K) have demonstrated that the migration of radicals proceeds at least down to 35 K and most probably even to lower temperatures. Therefore R_s characterises the value of $x_{act} \sim 10^{-3}$ – 10^{-2} rather than the degree of imperfection of the solid. (Migration of free radicals as such is not required for the correctness of the treatment of R_s values as a measure of the time-probability rather than space-probability of finding an active centre at any site in the solid. The equality $R_s = x_{act}$ which is the premise of following estimates can be fulfilled for any other mechanism of free valency migration—for example in cases of tunnelling of H-atoms and/or electrons which is certainly feasible even at very low temperature.)

Assuming that active centres are produced by ultraviolet radiation¹⁰ and that the density of quanta $\rho_{UV} \sim 10^{-2}$ cm⁻³, that is their flux $j_{UV} = \rho_{UV}c \sim 3 \times 10^8$ cm⁻² s⁻¹, we obtain the rate of absorption of ultraviolet quanta by interstellar dust molecules as 3×10^{-10} s⁻¹, so that the characteristic time between two successive ultraviolet absorptions by a molecule is $\tau_{UV} \sim 100$ yr. Meanwhile the fraction of time spent by any monomer molecule in its active state when it is able to participate in chain propagation is only

$\tau_{act} = x_{act} \tau_{UV} \sim 0.1$ – 1 yr. When $\tau_E > \tau_{act}$, the probability of formation of a polymer chain of ν links is

$$w_\nu = [\tau_{act}(\tau_{act} + \tau_E)^{-1}]^{\nu-1}$$

This expression decreases very fast with the increasing ν and therefore for the above-mentioned value of τ_E (20 K) $\sim 10^4$ yr, given by an Arrhenius-type extrapolation, the formation of even trimers is extremely improbable.

The validity of an Arrhenius-type extrapolation of data for $T \geq 77$ K to lower temperatures is highly suspect, however. In the conditions of our experiments⁵⁻⁹ when the dose rate of ^{60}Co γ radiation (which initiated polymerisation chains) was ~ 10 rad s⁻¹, the characteristic time for absorption of γ quanta was also close to $\tau \sim 100$ yr, and $\tau_{act} \sim 0.1$ yr. Even at temperatures as low as 4 K the length of polymerisation chains reached $\nu \sim 10^3$. This implies that the time for the addition of one new link to the growing polymer chain τ_0 had to be much shorter than 10^{-3} $\tau_{act} \sim 10^3$ – 10^4 s.

Indeed, our experiments⁵⁻⁹ have shown that the above-mentioned extrapolation of the average time τ_0 for the addition of one new link to the growing polyformaldehyde chain, based on Arrhenius' law, is absolutely inadmissible below temperatures around 77 K. The increase of τ_0 with the decrease of temperature in this region sharply slows down, and reaches an asymptotic plateau value: $\tau_0 \sim 10^{-2}$ s, below ~ 10 K with no further increase at lower temperatures. Such a value of τ_0 is ~ 5 – 6 orders of magnitude below the value of τ_0 necessary for the formation of chains with $\nu \sim 1,000$ at 4 K. This conclusion, based on direct laboratory data, has a very important bearing on the existence and formation of polymerised formaldehyde in interstellar space. A semi-quantitative explanation for the existence of a low-temperature limit to the rate of growth of chains in the polymerisation of formaldehyde was attained by using the concept of molecular tunnelling in exothermic chemical reactions¹⁴⁻¹⁶. As can be seen from all the above-mentioned considerations, there are now sufficient grounds to affirm that molecular tunnelling is the only mechanism which can provide the formation of long chains of polymerised formaldehyde in interstellar space.

It would seem most promising and worthwhile to analyse the possible role of molecular tunnelling in the general context of the evolution of prebiotic macromolecules and in the wider field of cosmic organic chemistry with reference to conditions of interstellar space, comets and the early Solar System as well as the primitive earth.

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Dust clouds and frictional generation of glow discharges on Mars

A REMARKABLE characteristic of those samples of the martian soil which have so far been analysed is the absence of carbonaceous matter down to the parts per billion (10^9) level¹. As well as a lack of endogenous organic material there is no sign of the component expected from infalling carbonaceous meteorites. The inorganic particles constituting the fine martian soil seem to be extremely 'clean'—far cleaner than terrestrial desert or Antarctic analogues. I suggest here that glow discharges generated by friction within dust clouds might explain this apparent absence of carbonaceous matter. In addition glow discharges might account for some reactions noted in the Viking biological experiments.

Fine siliceous material is frequently raised by Martian winds to produce dust clouds, sometimes in enormous quantities sufficient to shroud the entire planet². In the present cold, dry conditions³ it seems inescapable that frictional electrostatic charging will occur. On Earth we see natural examples of this triboelectric phenomenon in sandstorms and dusty volcanic eruptions³. Laboratory studies have confirmed that movement and dispersion of many types of finely-divided material result in the separation of charge and the generation of considerable electrostatic potentials⁴. It is not essential that the system be heterogeneous: homogeneous particles of different sizes can take up opposite polarities. The magnet experiments on the Viking landers have demonstrated a certain heterogeneity in the martian soil¹, however. Separation of the particulate components of a charged cloud can produce oppositely charged regions within it. At the pressures existing in the lower terrestrial atmosphere the potentials may be equilibrated by a spark discharge—the most dramatic example being volcanic lightning⁵. At reduced pressures, however, charge equalisation is facilitated by glow discharge⁶. Experiments⁷ show this mechanism to be most effective in the pressure range 0.1–10 torr: at the surface of Mars there is a dry 95% carbon dioxide atmosphere at a pressure of 5–6 torr (7–8 mbar; ref. 1). Studies of the glow discharge resulting from the generation of frictional electricity at low pressures were begun in the eighteenth century⁸, but frictional devices were soon supplanted by more reliable and controllable sources of high potential for the investigation of electrical discharges in gases. I therefore decided to check experimentally on the effect of agitating dry powders at reduced pressure.

A 5-l Pyrex flask was rotated manually about a hollow stub axle, two O-rings providing a vacuum seal. The speed of rotation controlled the magnitude and form of agitation of the dust from gentle sliding to a continuous cascade, an oscillatory motion proving especially effective. Evacuation was accomplished through the hollow axle by a conventional vacuum system incorporating gauges, a gas bleed, and a two-stage rotary pump. With air as the gaseous medium and fine sand (previously washed and oven dried) as the particulate material it was found that discharges visible in a darkened room were readily excited over a wide range of reduced pressures. At 0.1 torr a faint greenish-white glow extended for more than 5 cm above the agitated sand, being somewhat more intense adjacent to the rising wall of the rotating vessel. The system was then isolated from the pump and air allowed to enter as required through a needle valve. In the pressure range of particular interest (4–6 torr) the continuous glow contracted and brightened, and was accompanied by long, forked, ribbon discharges about 1 cm wide darting intermittently through the cascade of dispersed grains. Occasional intense spots of light on the surface of the moving sand gave a 'twinkling' appearance. At 10 torr ribbon discharges became narrower and brighter, but less frequent. This progression continued until at 50 torr only bright twinkling discharges remained.

It is recognised that the glow discharge is a very effective scavenger of even the most tenacious traces of organic matter from surfaces (particularly silicates) because cleaning with such a discharge is the conventional final stage in preparing an as-

tronomical mirror for aluminising⁹. The mechanism is primarily that of ion bombardment, but reactive intermediates produced in the gas phase may also be important¹⁰. It is therefore suggested that the fine, near-surface martian soil will have been subjected to repeated dissemination by seasonal winds and, while within these clouds, the separated particles exposed many times to the cleaning action of glow discharges. It will be noted that this process could operate discontinuously throughout an extended period of time right up to the present. It also differs from irradiation by solar ultraviolet in that the latter could not penetrate beyond the surface of a cloud or the undisturbed soil: the two mechanisms may be complementary.

In addition to the cleaning action, it is relevant to consider the accumulated chemical effects which may be produced by glow discharges⁷. It has been shown that they promote dissociation of molecular gases such as carbon dioxide, and can act as effective generators of ultraviolet light, ions, free radicals, atomic oxygen and ozone^{11,12}. The last two in particular could persist for varying lengths of time after the discharge, and diffuse to subsurface locations in the grains to exert powerful oxidising effects. Potential products include peroxide bonds, percarbonates (which evolve oxygen and carbon dioxide on gentle warming, and oxygen on addition of liquid water) and hydrogen peroxide addition compounds¹¹. It is therefore suggested that the widespread reddish coloration of the martian surface¹, as well as the unexpected reactions observed in the Viking biology experiments¹³, could in part be due either directly or indirectly to glow discharges. Living microorganisms (including spore-formers) do not long survive exposure to glow discharges and their products¹², so on this basis the fine, red, surficial martian dust would seem unfavourable for life-detection experiments. A better sampling scheme might be coring in the postulated permafrost terrain². It is perhaps ironic that in different conditions (notably in the presence of water) glow discharges can promote the abiogenic synthesis of organic molecules⁷.

Finally, although it is not expected that glow discharges on Mars would be detectable from orbit (much less from Earth), it would seem worthwhile to attempt radiofrequency and electrostatic field measurements on future landings.

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Daytime ionosphere scintillation associated with geomagnetic storms

EQUATORIAL spread F, although a night-time phenomena, has been reported on a few occasions during the dawn period¹. This is thought to be due to the remains of irregularities produced during the night. Earlier results have also shown that ionospheric scintillations and spread F have

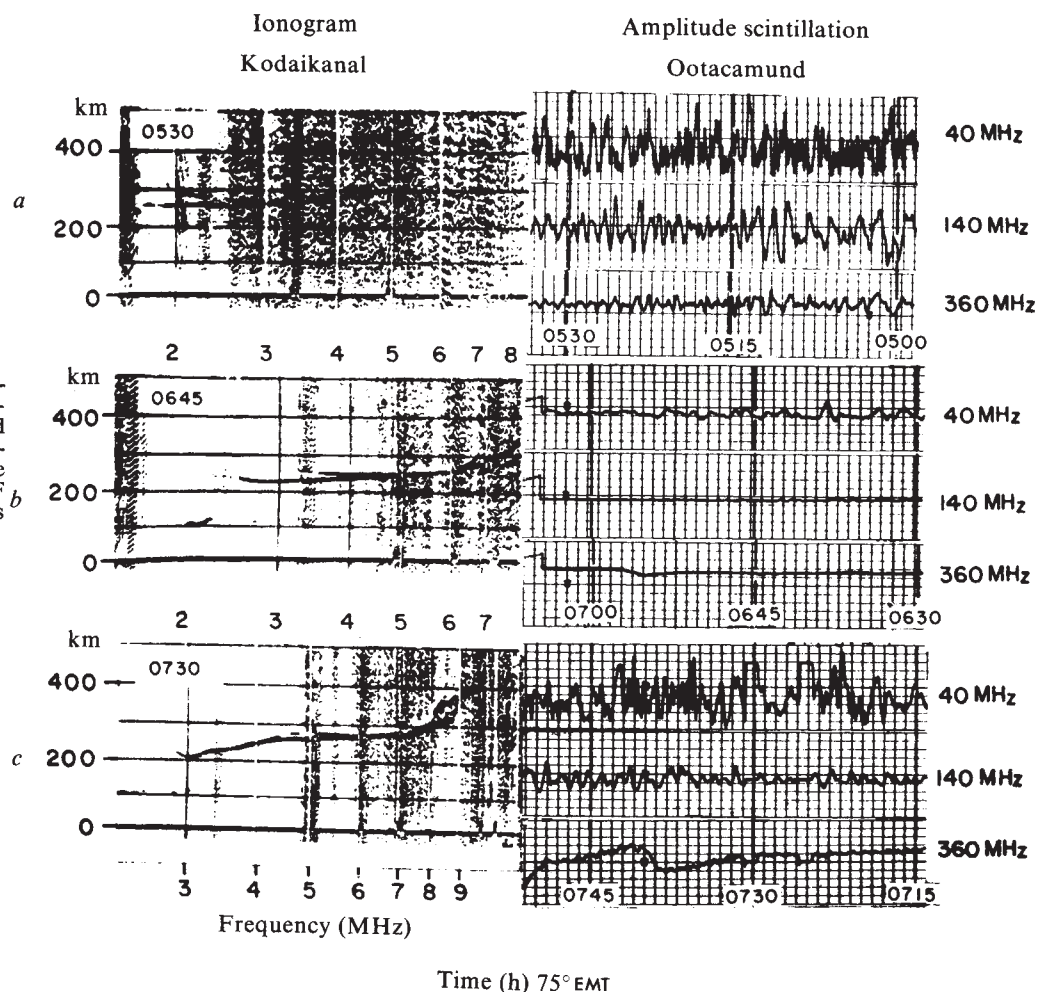


Fig. 1 Ionograms taken at Kodaikanal and the corresponding amplitude recordings of 40-, 140- and 360-MHz radio beacons at Ootacamund on 29 October 1975. Note the association between spread F and ionospheric scintillations specially during daytime (c).

a good correlation. These scintillations rarely occur between 0200 and 0800 LT, or around sunset hours². The present article deals with a unique observation of the equatorial region, where the irregularities produced during the night have vanished, and started again after sunrise. The fresh irregularities caused spread F and ionospheric scintillations at 40, 140 and 360 MHz. These irregularities can be associated with a geomagnetic storm.

Figure 1 shows the sample records of the amplitudes of 40-, 140- and 360-MHz beacons as received at Ootacamund (11°4'N, 76°7'E) at 0530, 0645 and 0730 LT (75° EMT). To monitor the ionospheric condition we have compared these records with the corresponding ionograms taken at a neighbouring station Kodaikanal (10°2'N, 77°5'E). During the pre-dawn period on 29 October 1975, both the spread F and the scintillations were quite strong. This is shown by Fig. 1a; the scintillation index at 40 MHz was 97%. The spread F as well as the scintillations had disappeared after 0603 LT and one can see in Fig. 1b for 0645 LT, clear F traces and fairly smooth amplitude records. There were no more scintillation until about 0712 LT when there was sudden onset on all frequencies and these scintillations continued until 0848 LT. Figure 1c for 0730 LT shows spread F near the critical frequency together with scintillations on all the frequencies. No sporadic E irregularities were seen on the ionogram, thus, the scintillations were caused only by F region irregularities.

The Indian Institute of Geomagnetism, Bombay, had observed a gradual commencement type geomagnetic storm at 1800 LT on 28 October 1975. Although the planetary geomagnetic indices were not high on 29 October 1975, the

equatorial D_{st} values showed an increase from 1400 UT (1900 LT) to 1800 UT (2300 LT) and a sudden drop afterwards reaching a minimum around 0800 UT (1300 LT). Thus the present observations seem to have occurred during the main phase of a strong geomagnetic storm.

The Faraday rotation angles were recorded at the same time as the amplitudes at Ootacamund. Using these data, total electron contents (TECs) at intervals of 5 min were computed. There were wave-like fluctuations in the TEC in the period of about 10 to 15 min.

Equatorial ionospheric scintillations in the early hours after sunrise is rare. Further, in the present case, the onset at 0712 LT was preceded by a no-scintillation period. Thus the scintillations around 0730 LT were not due to irregularities remaining from spread F of the previous night but were due to newly generated irregularities in the F region.

Various theories^{3,4} have been proposed for the generation of spread F near the magnetic equator during the post sunset period. The example shown in Fig. 1c is an abnormal case and cannot be explained by normal features of the F region assumed in most theories. It is likely that the F region irregularities discussed here were associated with the geomagnetic storm prevailing during that period.

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Volcanic dust veils and North Atlantic climatic change

ONE possible climatic control that has been neglected in empirical studies until recent years is the variation in the volcanic dust loading of the upper atmosphere. I shall discuss here the nature of the relationship between volcanic activity and the climate of the North Atlantic sector and its importance in relation to other proposed climatic controls on time scales up to 300 yr. Fluctuations on these time scales in a volcanic dust veil index (DVI) compiled by Lamb¹ and in parameters representative of the strength of the North Atlantic westerly winds and the temperature of central England are shown to be similar.

The DVI is an estimate of the amount of dust in the stratosphere injected by explosive volcanic eruptions. How the amount of dust input from an eruption is assessed depends on the type of observations available. The three methods generally used are based on the greatest depletion of the direct solar beam in middle latitudes, the estimated volume of solid matter dispersed as dust, and the lowering of the average air temperature in middle latitudes by the eruption. All available estimates are finally averaged (Lamb¹ gives further details). The estimation methods give rise to a degree of climatic dependence in the DVI which should not be ignored. The present record contains no assessments based on temperature alone. The atmospheric circulation indices used are temperature-independent.

To examine relationships between volcanic activity, the atmospheric circulation and climate of the North Atlantic

sector on timescales of 50 yr or more, non-overlapping 25-yr averages of the DVI, central England temperature² (CET) and a record of south-westerly surface wind frequency at London (B. M. Gray, East Anglia, unpublished data; pre 1728 figures are estimates) are used (Fig. 1). The latter is related to the character of the circumpolar westerly upper air flow as it crosses the North Atlantic, where it is determined by the strength, position and orientation of the Canadian upper air trough. These series were selected as they are the longest high resolution records of the atmospheric circulation and climate available for the North Atlantic sector. The degree to which they represent global events is difficult to assess in view of the limited availability of data for other sectors. Trends in CET over the past century are, however, similar to those observed in estimates of global or hemispheric temperature.

Table 1 lists correlation coefficients between the non-overlapping 25 yr averages of the three records for the period 1725 to 1950. The strongest relationships are between the DVI and the south-westerly frequencies (about 70% of the variance of the annual south-westerly record is explained by the DVI) and between the south-westerly frequencies and CET (about 80% of the CET_w variance explained by the winter south-westerly frequencies). The input of large amounts of volcanic dust into the stratosphere affects the amount and distribution of solar energy reaching the Earth's surface, thereby influencing the strength of the atmospheric circulation. Lamb¹ showed that the frequency of the North Atlantic upper air westerly winds reduces on a year-to-year timescale following large explosive volcanic eruptions. The strength of these upper air westerlies, and the associated surface advection of air from southerly, oceanic sources, is a major factor in determining the winter temperature in central England and Western Europe.

Table 1 Correlation coefficients for the period 1725–1950 (nine pairs of non-overlapping 25 yr averages).

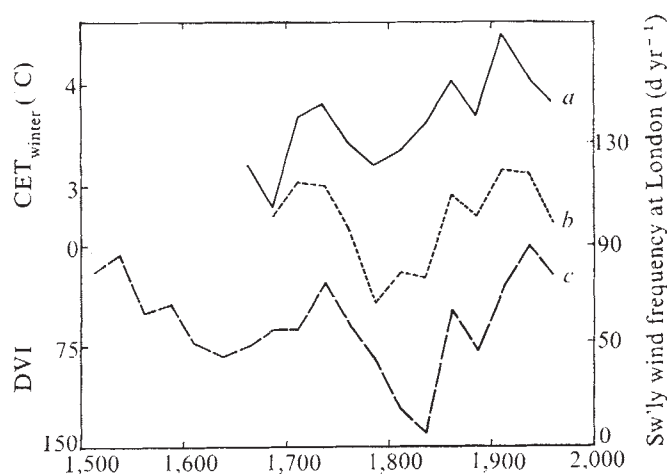
	DVI	CET _{μl}	CET _{winter}
DVI		−0.67*	−0.64
South-westerly frequency (annual)	−0.85*	+0.61	+0.86*
South-westerly frequency (winter)	−0.76*	+0.55	+0.90*

*Statistical significance above the 95% level.

Figure 1 shows that three major periods of stratospheric dust clearance have occurred on timescales of 50 yr and more, in the early sixteenth, eighteenth and twentieth centuries. These periods correspond to the maxima in the extended record of CET constructed by Lamb³ from historical and botanical evidence. The effects of a minor clearance in the second half of the nineteenth century are also clear in the CET_w and south-westerly frequency records (Fig. 1). Because of the relative shortness of the DVI record, the 200-yr interval between times of major dust clearance is considered fortuitous rather than evidence of a predictable cycle. A climatic cycle of about this length has, however, been frequently linked to solar variation^{4,5}. Roosen *et al.*⁶ proposed that this periodicity might result from regular changes in the moon's orbit. The associated changes in tidal stress could trigger volcanic activity and hence affect climate.

The varying level of volcanic activity explains much of the variation in CET on time scales of 50–300 yr but certain fluctuations present in CET are not seen in the DVI. Although the 200-yr periodicity is present in all three records, only CET_w contains a linear trend (Fig. 1). This trend results from the recovery from the depth of the Little Ice Age which occurred during the second half of the seventeenth century. Neither the magnitude of the DVI, nor the reduction of the south-westerly wind frequencies is sufficient to account for this climatic severity. Although all three records must be considered suspect during this period, another climatic control, perhaps affecting

Fig. 1 25-yr Averages of *a*, winter temperature in central England; *b*, south-westerly wind frequency at London, provisional values pre-1728; *c*, volcanic DVI.



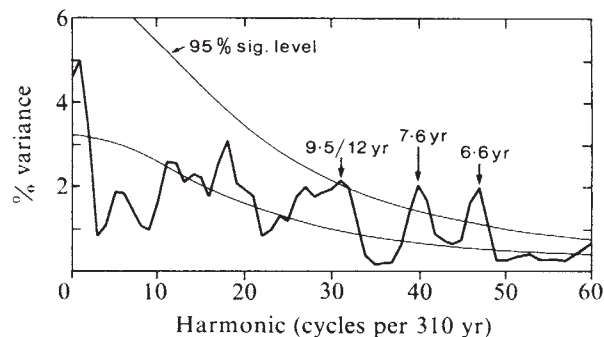


Fig. 2 Variance spectrum of the DVI, data for period 1500-1966. Calculated by taking the Fourier transform of the autocorrelation function, truncated at one-third of the data length. Red noise null continuum assumed as lag one autocorrelation coefficient significantly different from zero.

some other component of the atmospheric circulation, is required to explain the severity of this climatic shift. The low level of solar activity during the Maunder Minimum⁷ of the seventeenth century may have been responsible. Solar particles emitted during solar flares seem to influence the strength of the meridional component of the North Atlantic atmospheric circulation^{8,9}, although the mechanism is unclear. The climatic effects of changes in the meridional circulation of the atmosphere are generally obscured as the zonal circulation exerts a stronger control over the UK climate⁹. Schneider and Mass¹⁰ have modelled fluctuations in global temperature since 1600 employing fluctuations in volcanic and solar activity as climatic controls. In addition to solar variability, human activity may be a significant factor from the mid-twentieth century onwards.

Explosive volcanic eruptions inject into the stratosphere large quantities of volcanic dust which slowly falls out over succeeding years. This gives the annual DVI record a saw-toothed appearance: a series of impulses which gradually decay. Because of the damped response of the atmosphere-ocean system, a succession of fairly regular volcanic eruptions may result in an apparently cyclical climatic fluctuation. If volcanic eruptions occur with approximate regularity, a corresponding peak should appear in the variance spectrum of the DVI. If volcanic dust is an important climatic control, a similar peak may also appear in the spectra of climatic data for the same period. Matching peaks, however, do not necessarily show that volcanic activity and climate change in a regular, predictable cycle, the DVI periodicity may just be a chance characteristic of the data interval employed.

The variance spectrum of the entire DVI record is shown in Fig. 2. Three peaks are significant at the 95% level: at 9-12.5 yr, 7.6 yr and 6.6 yr. The peak at 9-12.5 yr is broad and, therefore, represents a process of highly variable period. It will not be discussed further. Bandpass filtering indicates that the two cycles

at 7.6 and 6.6 yr are present through most of the period 1500 to 1966 but of low amplitude during the eighteenth century. They interfere to produce an amplitude modulated cycle with a wavelength close to 7 yr. The interval between periods of maximum amplitude is about 50 yr. The frequency and phase of the cycle are also variable. The fact that the parameters of the cycle (amplitude, phase, period, etc) are not constant places a considerable restriction on its forecasting potential.

The *P* index is a measure of the mobility of the flow of the atmospheric circulation over the UK on a day to day time scale¹¹. In general, a high value of the *P* index indicates a period of mobile, westerly and cyclonic circulation types associated with vigorous North Atlantic westerlies. The variance spectra of this index and the DVI, calculated from available data for the period 1850 to date, are shown in Fig. 3. Both spectra contain a statistically significant peak at 7-8 yr. Considering the resolution of these spectra, the two peaks are indistinguishable in period. The variance spectrum of the CET record for the period 1816-1973 contains a similar peak but the earlier section of the record (1659-1815), comprising the eighteenth century when the 7 yr cycle in the DVI was of low amplitude, does not. Berlage¹² has discussed the apparently global extent of the 7-yr climatic cycle which is particularly marked in the El Niño phenomenon on the coast of Peru. However, many claims made for climatic cycles of any period are based on doubtful statistical analysis and require re-examination with modern techniques.

The volcanic cycle of 7 or 8 yr, and the associated climatic variation, may possibly be forced and, to a certain extent, predictable, as a similar fluctuation is present in parameters of the earth's rotation. Anderson¹³, and Press and Briggs¹⁴, have suggested that variations in seismicity, the two components of the earth's rotation (the rotational speed and polar motion) and the atmospheric circulation (and climate) could be linked. A fluctuation of 7 or 8 yr has been observed in modern measurements of the Earth's rotational speed¹⁵, although the data interval is insufficient to test its statistical significance. Interaction between the annual component of the polar motion, forced by seasonal redistribution of the atmospheric mass, and the Chandler Wobble period of about 14 months gives rise to an approximately 7-yr fluctuation in the polar motion. The amplitude of the annual component also undergoes a 7-yr fluctuation¹⁶. It has been claimed that the polar motion could have a tidal influence on the atmosphere¹⁶. It is dangerous to discern cause and effect in such fragmentary evidence but the possibility that volcanicity, seismicity, the atmospheric circulation and the earth's rotation form a complex, interactive loop with a characteristic timescale of about 7 yr, perhaps determined by the period of the Chandler Wobble, cannot be neglected.

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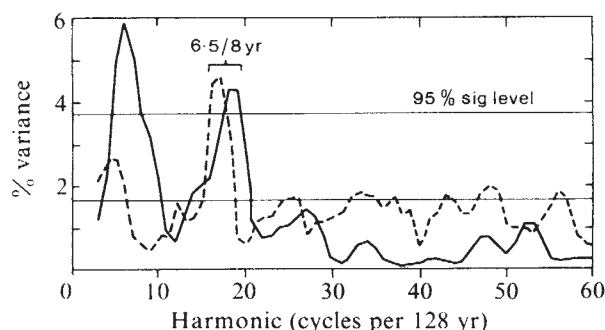
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Fig. 3 Variance spectra of the DVI (—), data for period 1850-1966, and the *P* index (---), data for period 1861-1971. Calculated using the fast Fourier transform, to obtain similar resolution and avoid data loss.



Oxygen isotope stratigraphy of Late Pleistocene coral terraces in Barbados

DISPUTE over the correlation of the last interglacial event in deep-sea cores with the corresponding shoreline feature on islands preserving fossil coral terraces has become a fundamental stumbling-block to progress in Pleistocene geochronology. If polled independently most scientists working in this area would agree that the last interglacial event is clearly recorded in their own data, yet the problem of precise correlation among the various types of data has remained the subject of controversy for the past eight years¹⁻⁸. By making oxygen isotope determinations in molluscs collected from the Late Pleistocene coral terraces in Barbados, we have directly correlated these terraces with the oxygen isotope stratigraphic record of deep-sea cores, and have established that Barbados terraces I, II, and III (ages 82,000, 105,000 and 125,000 yr BP) were all formed during stage 5 of the standard oxygen isotope stratigraphy.

We will first review the data from interglacial highstands, and then that from interglacial sections in deep-sea cores. Pleistocene beach deposits are widespread on all the continents and on many islands; of particular interest are the latitudes of coral growth, within which Pleistocene sea-level stands are marked by fossil coral terraces. Aragonite coral skeletons are amenable to absolute dating using both the $^{230}\text{Th}/^{234}\text{U}$ method⁹ and the $^{231}\text{Pa}/^{235}\text{U}$ method¹⁰ within the time range Recent to ~150,000 yr BP. A large number of localities possess coral reef terraces which yield uranium-series dates in the region of 125,000 yr BP^{4,11,12}. In tectonically stable areas, such as the Bahamas¹³, Hawaii¹⁴ and elsewhere, the 125,000 yr old terrace is found several metres above present sea level, and is the youngest pre-Holocene terrace present⁹. These data indicate that the 125,000 yr BP sea was above the present sea level, and that since that time the sea has been continuously below its present level.

Coral reef sequences from tectonically emergent areas support the inference that subsequent sea level maxima have not reached the present level. For example, on Barbados, the Barbados III shoreline (125,000 yr BP) is well mapped and abundantly dated. Owing to tectonic uplift, the feature is presently at elevations ranging from 20 to 60 m; at lower elevations the Barbados II and Barbados I terraces are also mapped and dated². Sea levels considerably below the present level have been estimated for these times by assuming a constant uplift rate calibrated by the present position of the Barbados III terrace. Similar studies in New Guinea on the tectonically emergent Huon peninsula lead to the same conclusion¹⁵, as do studies in the Ryukyu Islands¹¹. This implies then that the 125,000 yr BP terrace records the last interglacial in the sense that it formed the last time that continental ice volume was as small as it is today.

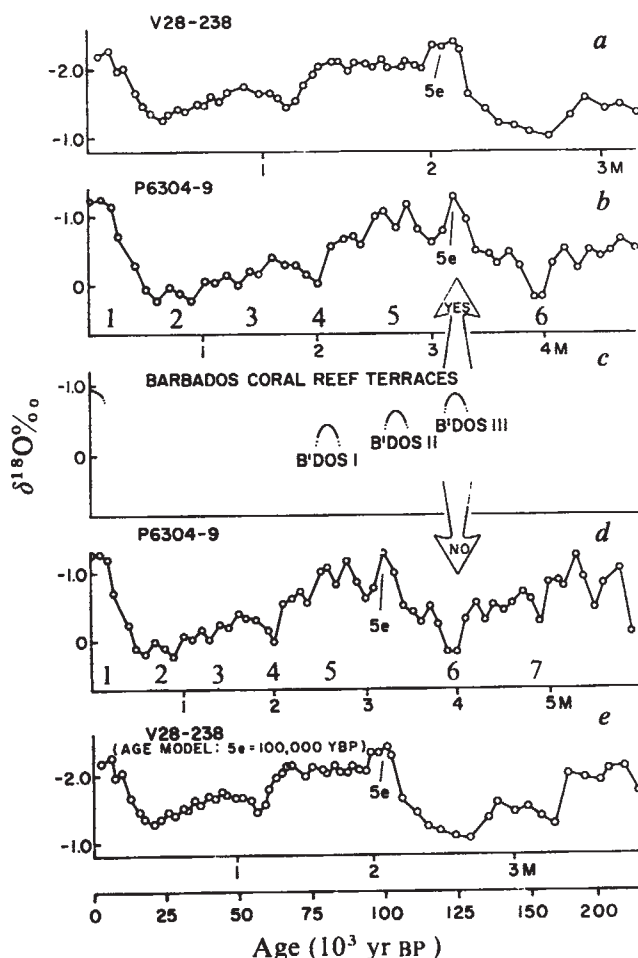
In deep-sea sediments, the oxygen isotopic composition of calcareous foraminifera has become a widely used stratigraphic tool. The oxygen isotope record has been divided into isotope stages whose boundaries can generally be easily recognised in all oceans¹⁶. Stage 5 was the last predominantly interglacial stage^{3,4,17}, within which the earliest substage 5e may probably be correlated with the last interglacial in a more strict sense as used, for instance, by palaeobotanists³. The stage 6-5 boundary, also known as Termination II⁴, has been described as the most widely correlatable horizon in the whole geological column¹⁸. It is the age of this clearly-defined horizon, or of the equally clear interglacial event which followed it, that is a matter of contention.

One very persuasive line of reasoning, stems directly from the interpretation of the oxygen isotope record. It has been argued that the changing oxygen isotopic composition which is observed in foraminifera from deep-sea sediment cores, stems almost entirely from changes in the oxygen isotopic composition of the ocean itself, resulting from the storage of isotopically light water in the continental ice sheets^{16,19-21}, and hence may be an ice-volume or sea-level record. Clearly, if this is a correct interpretation, then there can be no doubt as to the correlation of the Barbados III

terrace, with the earliest part of stage 5, which was uniquely interglacial in isotopic terms³. There are, however, reasonable arguments in favour of a smaller contribution to the oxygen isotopic record from this source²², and if they are followed then the correlation of the Barbados III coral terrace with the early part of stage 5 is not a unique solution.

An alternative approach, of course, is the dating of the marine sediments themselves; this is the chief source of evidence for an alternative chronology. Beyond the range of ^{14}C dating, the uranium decay series may be used to date marine sediments. The application is less simple, however, than in the case of coral dating; there are difficulties in the analytical methods and acute problems regarding the interpretation of the measurements²⁴ which are widely acknowledged to exist. Several cores have yielded apparently consistent data which clearly point to an age near 90,000 yr BP for substage 5e²⁵. Another core has been studied in considerable detail, and has consistently yielded an age near 120,000 yr BP for the same event^{2,4,27}. Few other cores have been analysed in sufficient detail to contribute to a solution of the problem, and interlaboratory comparisons have so far only clouded the issue²⁸. Thus, if correlation is to be based only on an absolute age estimation, a case may be made for correlating substage 5e either with the Barbados II or the Barbados III terrace.

Fig. 1 Oxygen isotope stratigraphy of Barbados coral reef terraces (c) compared with oxygen isotope stratigraphy of Caribbean core P6304-9 (b and d) and of equatorial Pacific core V28-238 (a and e). All five are plotted against a time axis; for the cores, depth in metres is also indicated. a and b are plotted assuming that the age of substage 5e is about 125,000 yr BP; d and e are plotted assuming that the age of substage 5e is about 100,000 yr BP. In c, the oxygen isotope data from this paper are plotted at the ages determined for the coral terraces from which our samples were collected. Data for a and e in refs 3, 4, 17 for b and d from ref. 31; for c from Table 1. The alternate timescales indicated respectively by a and b, and by d and e, are those depicted in Fig. 4 of ref. 8.



This dilemma, as well as other less substantial evidence, led Emiliani and Shackleton⁸ to publish their infamous 'double curve' in which generalised versions of the isotopic record as a function of time were sketched for two different timescales through the last interglacial.

Clearly, the correct way to solve this problem is to make a direct stratigraphic correlation between the coral reef sequence and the deep-sea sediment sequence, instead of attempting correlation by means of age estimation. The coral terraces of Barbados contain molluscs which, like foraminifera, deposit their carbonate shells in oxygen isotopic equilibrium with sea-water²³. Thus, the oxygen isotope record in a mollusc sequence should, in principal, be almost identical with the sequence observed through isotopic analysis of planktonic foraminifera in deep-sea sediments from the nearby ocean floor. Specifically, molluscs in the Barbados III terrace should be 0.1 ± 0.1 per mil more isotopically negative than those living today, if correlation with 5e is correct³ and if 5e is therefore 125,000 yr old; they should be up to 1.8 per mil more positive if the terrace formed during the cold part of stage 6 (Fig. 1) and 5e is correlated with Barbados II with an age of about 100,000 yr; they should be about 0.9 per mil more positive, with the age of 5e being about 90,000 yr, according to the correlation shown by Emiliani and Rona (they remark that molluscs in some raised beaches do give isotopically positive ^{18}O values suggestive of a glacial age—see ref. 7).

Molluscs were collected by R. K. Matthews from Barbados terraces I, II and III during summer 1973. Care was taken to collect only from reef front localities (predominantly *A. palmata* and *A. cervicornis* facies). By specifically avoiding lagoonal materials, we avoid the anomalous salinity and temperature conditions characteristic of that environment. Modern subtidal molluscs from Barbados were collected alive specifically for this study by Finn Sander (Director of the Bellaires Research Institute, Holetown, St James, Barbados W1).

Our first isotopic measurements on these materials was made in Cambridge⁸ during 1974. Shells were sampled as described in ref. 30, and analysed in a VG-Micromass 602C mass spectrometer using the sample preparation system described in ref. 16. In the course of these determinations and subsequent investigation of the specimens in thin section, we became somewhat apprehensive about possible contamination by boring algae. We evolved a new sampling procedure. At Brown, molluscs were embedded in plastic, sectioned perpendicular to the shell wall and polished. An acetate film peel replica of the polished surface was prepared before and after sampling the shell with a small drill or with a tungsten carbide scribe. Examination of the peels with a light microscope allows detection of any areas of alteration or other abnormalities which may be avoided in sampling. Subsequent peels provide a permanent record of precisely what was sampled. We repeated the isotopic analyses at Brown University during 1975. The analytical methods used in the Benedum Stable Isotopes Laboratory at Brown University were similar to those used in Cambridge; again, a VG-Micromass 602C mass spectrometer was used. Results of these two independent studies are substantially in agreement. We report here only the second data set because we are confident that our new sampling procedure avoids contamination from boring algae.

Variation in average oxygen isotopic composition among Barbados Recent, Barbados I, II, and III are summarised in Table 1. We consider variation in average isotopic composition among these stratigraphic units to be primarily a measure of differences in global ice volume. We have, however, given detailed consideration to the possible contribution of temperature and salinity variations. The seasonal isotopic ranges measured are in good agreement with the range of values we observe in recent molluscs. The potential contribution of temperature and salinity to variation among the means is small. More importantly, these factors would also affect isotopic composition of planktonic foraminifera from tropical deep-sea cores. As this paper deals with correlation of Barbados terraces to deep-sea cores by means of oxygen isotope stratigraphy, the precise origin of the signal which is common to the two data sets is of secondary consideration.

Table 1 Average oxygen isotopic composition of Barbados molluscs from Recent and Pleistocene terraces. Values are reported in standard notation relative to the PDB standard²³

Stratigraphic level	$\delta^{18}\text{O}$ mean and standard deviation (‰)
Living Recent (17 analyses among 6 specimens)	-0.93 ± 0.26
Barbados I (82,000 yr BP) (23 analyses among 9 molluscs)	-0.41 ± 0.41
Barbados II (105,000 yr BP) (16 analyses among 6 molluscs)	-0.62 ± 0.40
Barbados III (125,000 yr BP) (16 analyses among 6 molluscs)	-1.04 ± 0.55

One sample taken from one of the Barbados III shells yielded a value of $+1.77\%$, well over three standard deviations from the mean. We have not included the measurement in estimating the mean for this terrace. The observed standard deviation derives partly from seasonal temperature variation at the site; if average temperature varied with a standard deviation of $\pm 1^\circ\text{C}$ this would give rise to oxygen isotopic composition varying with a standard deviation of $\pm 0.2\%$. Data will be submitted to the CLIMAP Data Base Management System at Brown University and subsequently archived with the National Geophysical and Solar-Terrestrial Data Center (NGSDC), Boulder, Colorado.

Figure 1 presents graphic comparison of the oxygen isotopic record of two deep-sea cores and the Barbados terraces in the time range 0–150,000 yr BP. Graphs *a* and *b* place isotopic substage 5e at 125,000 yr BP whereas graphs *d* and *e* place substage 5e at 100,000 yr BP. Graph *c* presents Barbados mollusc isotopic data from Table 1 and terrace ages as determined by ^{230}Th dating of corals from Barbados I, II, and III. Correlation of the Barbados data with graphs *a* and *b* is good, whereas correlation of Barbados data with graphs *d* and *e* forces correlation of isotopically light (interglacial) Barbados III with outrageously glacial conditions in deep sea cores. Thus, we conclude that isotope substage 5e must surely be correlated with Barbados III and must, therefore, in turn be correlated with the terraces elsewhere in the world which indicate a sea-level somewhat above present levels at around 125,000 yr BP. While geochemists may continue to discuss the relative precision of absolute age estimates obtained from coral terraces and from deep-sea cores, our data firmly establish the stratigraphic correlation between the two sequences.

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Biological markers in coal and coal liquefaction products

ISOLATION of saturated isoprenoids from coal liquefaction products is an indication of the remarkable stability of these compounds. In the present investigation we show that some 'biological markers' and asymmetric carbons can survive the conditions of coal liquefaction without rearrangement or complete racemisation. Also, odd carbon numbered *n*-alkanes do not predominate, as reported for some coals, in either the coal liquefaction products studied or the feed coal. The distribution of saturates in the synthetic oil is very similar to that of some petroleum¹. The detection of 'biological markers' in coal and coal liquefaction products supports the geological and chemical evidence that coal is a product of the progressive fossilisation of plant debris. Optically active materials isolated from peat, lignite, brown and bituminous coals corroborate this theory. Additionally, hydrocarbons bearing skeletal structures that are widely distributed among plants have been extracted from coal. Ōuchi and Imuta² have found normal alkanes from C₉-C₃₁ in the benzene extracts of Yūbari coal; more recently, Harrison³ and Bartle, *et*

*al.*⁴ have identified the normal alkanes and saturated isoprenoids in the supercritical toluene extracts of bituminous coals.

'Biological markers' are compounds which exhibit long term chemical stability to diagenesis and which also possess skeletal features related to biosynthetic sequences⁵. Compounds such as *n*-alkanes, iso-alkanes, anteiso-alkanes, isoprenoid alkanes, porphyrins, and long chain fatty acids show stability for long time periods in geological conditions and have been identified in coals^{4,6}, petroleum, oil shales, recent and ancient sediments¹, meteors, and oil sands. Also, the observation of optical activity among organic compounds is closely associated with their biosynthetic origins.

During an investigation of the saturate materials in coal liquefaction products, several classes of compounds that are biological markers were detected. The oil studied was produced by catalytic hydrodesulphurisation of coal at 450 °C and 300 atm pressure of hydrogen in a half-ton-per-day bench scale SYNTHOIL unit at the Pittsburgh Energy Research Center^{7,8}. In this investigation, the solids in the gross liquid product were removed by centrifugation in the SYNTHOIL plant and the resulting liquid subjected to solvent separation using benzene and hexane⁹ to yield a heavy oil constituting 60-70 wt % of the centrifuged liquid product. The heavy oil fraction was separated by the SARA¹⁰ (saturates, aromatics, resins, and asphaltene) technique to yield a saturate fraction which amounted to 6-7 wt % of the heavy oil. The saturate material was further separated on 5-Å molecular sieves¹¹ to yield *n*-paraffin and non *n*-paraffin fractions, 29 and 71 wt % of the saturate fraction, respectively. These fractions were then chromatographed and analysed by combined gas chromatography-mass spectrometry (GCMS).

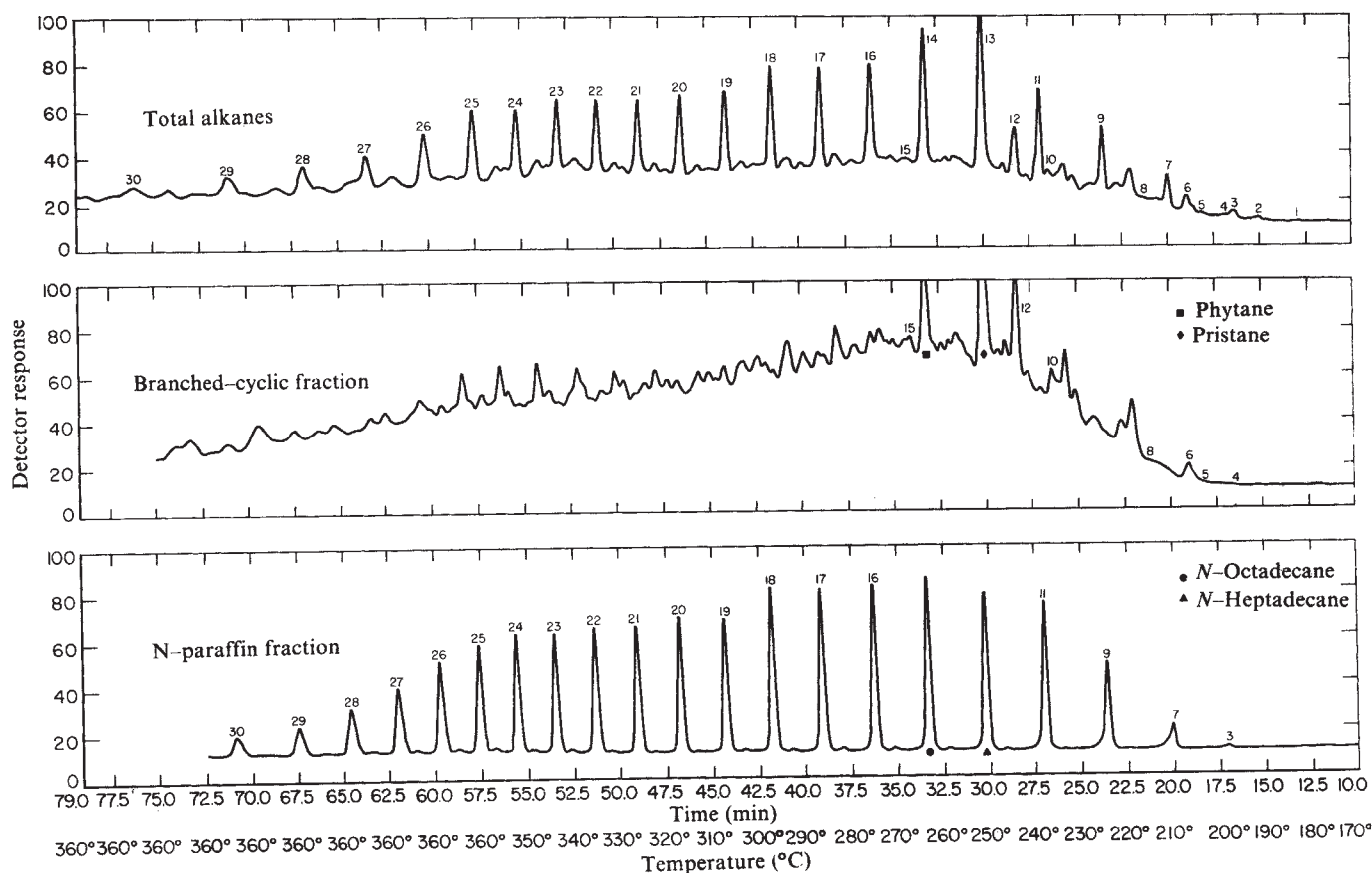


Fig. 1 The top chromatographic profile is of the total saturate materials isolated from coal liquefaction product. The middle and bottom chromatograms are subfractions of the total saturate materials separated by 5-Å molecular sieves. The combined GCMS analyses were performed with a DuPont 490 mass spectrometer interfaced to a Varian 1700 Series gas chromatograph, equipped with an 80:20 splitter and a flame ionisation detector, and to a Hewlett-Packard 2100A computer used for spectrometric data storage and reduction. The gas chromatographic separations were performed employing a 15 feet $\times$ $\frac{1}{8}$ inch stainless steel column packed with 5% Dexsil 300 on acid washed Chromosorb-G at a helium flow rate of 30 cm³ min⁻¹ and appropriate temperature programming conditions. Each of the above chromatograms were run in duplicate. In all cases, the two chromatograms of the same material were identical.

Table 1 Analyses of the total saturate materials from a coal liquefaction product

GC peak number	Compound*	saturates† (mg per g)
1	<i>n</i> -dodecane	trace
2	<i>C</i> ₆ -cyclohexane‡	trace
3	<i>n</i> -tridecane	4.3
4	<i>C</i> ₃ -decalin	0.7
5	methylethyldecalin	1.1
6	cyclohexylcyclohexane	3.9
7	<i>n</i> -tetradecane	8.7
8	methylcyclohexylcyclohexane	0.9
9	<i>n</i> -pentadecane	12.0
10	perhydro-3-ring	1.4
11	<i>n</i> -hexadecane	13.4
12	<i>C</i> ₁₈ -isoprenoid	6.9
13	<i>n</i> -heptadecane and pristane	50.1
14	<i>n</i> -octadecane and phytane	38.7
15	perhydropyrene	3.6
16	<i>n</i> -nonadecane	17.2
17	<i>n</i> -eicosane	15.5
18	<i>n</i> -heneicosane	15.7
19	<i>n</i> -docosane	14.3
20	<i>n</i> -tricosane	14.3
21	<i>n</i> -tetracosane	12.2
22	<i>n</i> -pentacosane	11.5
23	<i>n</i> -hexacosane	12.2
24	<i>n</i> -heptacosane	10.1
25	<i>n</i> -octacosane	10.7
26	<i>n</i> -nonacosane	10.4
27	<i>n</i> -triacontane	9.0
28	<i>n</i> -hentriacontane	8.9
29	<i>n</i> -dotriacontane	5.3
30	<i>n</i> -tritriacontane	3.4
		316.4 mg

*Personal communication, D. M. Jewell: many of the low boiling materials are lost during the solvent removal steps of the SARA fractionation. The loss is essentially zero for hydrocarbons of 15 carbons and above.

†The quantity of the various hydrocarbons listed is approximately within $\pm 20\%$ of the amount shown.

‡Indicates the number of alkyl carbons as substituents on the ring.

The chromatographic profiles of the saturate materials isolated from the coal liquefaction products before and after separation into two fractions on 5-Å molecular sieves are shown in Fig. 1; the identifications of the various numbered chromatographic peaks are given in Table 1. Inspection of the chromatographic profile of the *n*-paraffin fraction from the coal liquefaction product indicates

this predominance. Preliminary data indicate that the distribution of *n*-alkanes in this coal liquefaction product is similar to their distribution in some petroleum¹.

A sample of the feed coal, 364 g, used in the liquefaction process (Ireland Mine, W. Va., hvAb) was extracted in a soxhlet using benzene, in order to show that some of the *n*-paraffins found in the liquefaction product were originally in the feed coal. The benzene was removed from the extract by freeze drying, yielding 1.72 g of solids. This solid was batch extracted with *n*-pentane and centrifuged, yielding 1.29 g of pentane soluble material. The pentane soluble material was batch washed through silica gel (Brockman Activity I, 70–230 mesh), yielding 0.33 g (0.99 wt % of original coal) of non-silica gel-absorbed material. The pentane-soluble, non-silica gel-absorbed material was analysed for the *n*-paraffins by combined GCMS. The chromatographic profile and identification of the normal paraffins is shown in Fig. 2. Inspection of this chromatogram indicates that odd carbon numbered *n*-paraffins do not predominate over even. The *n*-heptadecane peak also contains pristane. Ōuchi and Imuta's² data, CPI ~ 1.09 , indicate that there is no predominance in Yübari coal and thus, is in agreement with the results obtained in this investigation, while the recent work of Bartle, *et al.*⁴ indicates that there is a predominance of odd over even carbon numbered *n*-paraffins in the bituminous coal they studied. Leythaeuser and Welte^{1,3} have clearly shown that, in the case of coals from the Upper Carboniferous of the Saar district, the predominance of odd over even carbon numbered *n*-paraffins decreases with increasing coal rank. This explains why the results among different workers concerning the predominance of odd over even carbon numbered *n*-paraffin homologues in coal seem to disagree. Bartle, *et al.* investigated a low rank coal (NCB Coal Rank Code 802) while the coals investigated here and by Ōuchi and Imuta were high rank bituminous. The work of Leythaeuser and Welte included only coals of specific European origin and, therefore, a more comprehensive study involving many coals from different continents is needed to determine whether the correlation between *n*-paraffin distribution and coal rank can be generalised.

In addition to *n*-alkanes, discussed above, another class of biological markers found in coal liquefaction products is the saturated isoprenoids—pristane, phytane and a *C*₁₈ isoprenoid were identified. The precursors of these hydrocarbons are unknown, but it is surmised that they result from the diagenesis of phytol, a moiety widely distributed among plant pigments. Phytane and pristane are known to be in coals^{2,3}. Thus the saturated isoprenoids and normal paraffins found in coal liquefaction products may be from two sources: (1) formed during the liquefaction process from a precursor having the same or similar

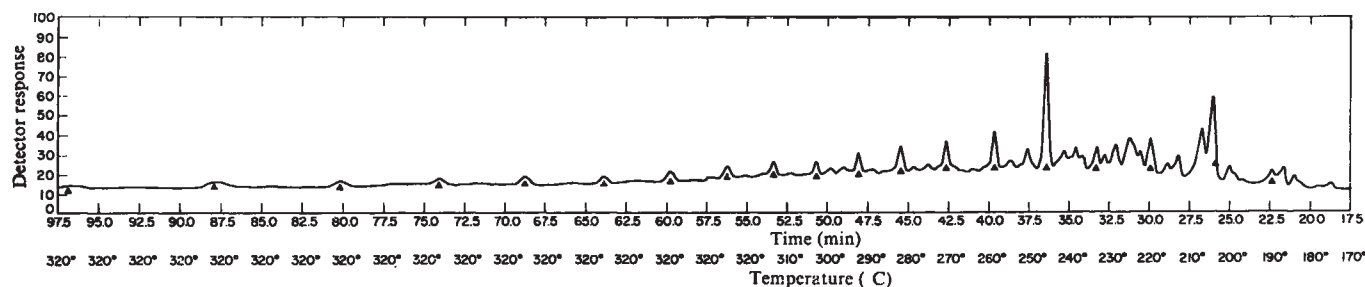


Fig. 2 Chromatographic profile of the pentane-soluble, non-silica gel absorbed material obtained from the benzene extraction of hvAb coal. The starred peaks indicate the *n*-paraffins. The first such labelled peak is *n*-tridecane, the second is *n*-tetradecane, and so on. The analysis was performed using the same equipment and conditions described in Fig. 1. This material was gas chromatographed twice to minimise errors in sample handling. Both chromatograms were identical.

that the odd carbon numbered *n*-paraffins do not predominate over the even. The carbon preference index¹, CPI, calculated using mole per cent data obtained from the chromatographic profile of the *n*-paraffin fraction equals 1.08. The *n*-paraffins are equally distributed between odd and even carbon numbered homologues. Others^{1,2} have shown that thermal alteration of organic sediments causes elimination of any carbon numbered preference. Thus, if the feed coals contained *n*-alkanes having a large predominance of odd carbon numbered homologues, liquefaction could eliminate

carbon skeleton, or (2) are present in the feed coals as entrained alkanes extractable by benzene, survive the liquefaction process, and go into the liquefaction product.

The optical activity of the saturate fraction from a coal liquefaction product was investigated and was found to rotate plane polarised light, $[\alpha]_D^{25} = +0.340^\circ$. The low degree of optical rotation is possibly a consequence of the partial racemisation of many enantiomers during the liquefaction process. It is interesting that the saturate fraction was dextrorotatory. Zahn, *et al.*^{14,15}

showed that the distillates from coal hydrogenation products and carbonisation tars from Pittsburgh seam coals showed positive rotation only. Hydrogenation oils produced from other coals displayed various positive and negative rotations but the distillates having positive rotation were far more numerous than those having negative rotations^{14,15}. Mair¹⁶ has made the same observation concerning petroleum distillates.

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Stratigraphy of buried soil at Teindland Forest, Scotland

EDWARDS *et al.*¹ have described and discussed new palynological and fabric analyses which have made an important contribution to our understanding of the stratigraphical history of the unique buried soil at Teindland, first described and dated by Fitzpatrick². Since 1962 detailed information relating to the distribution and periglacial modification of glacial deposits in the vicinity of Teindland has been gradually accumulated during the soil survey of the area represented on Sheet 85 (Rothes)³. Here we relate these field observations to the stratigraphy of the buried soil section at NJ 297570, and obtain a rather different interpretation of the nature of the 'sandy till' described by Edwards *et al.* as overlying the buried soil, and of the general stratigraphy of this site.

The summit area of Teindland Forest and the east facing slopes are covered by till and solifluction deposits derived from sedimentary rocks of Middle and Upper Old Red Sandstone age with some admixture of acid schists in the till at the southern end of Teindland Forest. Along the lower edge of the east facing slope there are scattered remnants of sandy and gravelly lateral moraine mostly lying above the line of the B9015 road. Between junctions at NJ 311522 and NJ 309550 this road also marks the approximate upper edge of the highest fluvioglacial terrace of the Spey Valley.

Above about 185 m no frost cracks are present in the compact red till exposed in profile pits and natural sections. On the eastern sideslopes the till is generally overlain by a very variable blanket of coarse textured stony solifluction deposits and below 120 m distinctive grey frost cracks with ochreous edges are present in the till. This distribution pattern has been confirmed at Wood of Cairnty on the east side of the Spey Valley. In sections alongside a forest track at about 165 m only the remnant basal tips of frost cracks can be seen, whilst downslope in a roadside cutting at about 125 m well-developed frost cracks are present in red till which is buried below a surface layer of solifluction debris. Most of the localities around Teindland where frost cracks or wedges have been recorded during soil survey are listed in Table 1.

From the field evidence it is clear that at some time after the deposition of the local red till there was a period of intensely cold periglacial climate during which a fairly uniform pattern of frost cracks and wedges was developed over the local landscape. Subsequent denudation of the hilltops with downslope migration of solifluction debris was not necessarily restricted to one episode. There is considerable evidence^{4,5} for widespread solifluction and erosion in Scotland during the period of the Loch Lomond Readvance (Pollen Zone III)⁶ and local evidence of some later erosion in the form of footslope accumulations of sandy gravel and colluvium above a peat band containing a late Sub-Boreal pollen assemblage at Bogincur farm (NJ 307554).

The pit face at Teindland has eroded considerably since it was first described. As exposed in 1962 the organic horizon, subsequently ¹⁴C dated at 28,140±480 and -450 years b.p. (NPL-78), had the form of a solifluction lobe cut through by a number of stepped frost cracks (Fig. 1). Only a limited part of the buried organic A horizon remains and on the longer backslope face of the pit the buried soil is represented by a layer of yellow-brown sand derived from the B horizon.

Thin sections prepared from the organic horizon in 1965, 1967 and 1976 consistently show that the organic material is heavily charred and contains coniferous charcoal closely resembling *Pinus sylvestris* charcoal prepared from wood recovered from a 'Grentz' horizon⁷. The presence of (burnt out) pine wood in this context may be indicative of the onset of colder climatic conditions⁸. The charred nature of the organic horizon and the distinctive sandy infill of the cracks suggests that the sand above the organic horizon was deposited soon after the fire. Photographs taken in January 1977 show clearly that this sand layer constituted part of the curved front of a solifluction lobe low down on the eastern side of the present section and was therefore deposited before this lobe formed. The size of the frost crack steps relative to the size of the solifluction lobe (Fig. 1) indicates some movement of the lobe before as well as after the formation of the cracks, so that permafrost was already present before the cracks formed. West⁹ has noted that temperatures around -6 to -8 °C, considerably lower than the temperature required for the promotion of permafrost, seem to be necessary for the formation of frost wedges. The surviving part of the buried H horizon seems to owe its preservation to rapid burial (at a break of slope) under sand eroding from the A and B horizons of the surface immediately upslope, a cover which was rapidly consolidated by the subsequent rise of the permafrost table. The material overlying the organic layer, described as 'sandy till' by Edwards *et al.*¹, is similar to but slightly coarser in texture than, the red till generally encountered in profile pits close to this site (Table 1). The very variable nature of this deposit which has been revealed over a period of years and the included irregular sandy lenses are not typical of the local red till. The

Table 1 General distribution of periglacial phenomena around Teindland, range in clay content of the local red till and clay content of soliflucted till above the approximately 28,000 years b.p. organic horizon at Teindland.

Site name	Description	Grid reference	Altitude* (m)	Presence/absence of frost cracks/wedges	Clay content of till (%)
Findlay's seat no. 1	Profile pit	NJ 283 537	260	Frost cracks absent	10–16
Teindland forest no. 1	Profile pit	NJ 289 548	240	Frost cracks absent	11–17
Teindland no. 2	Profile pit	NJ 300 529	185	Frost cracks absent	≈ 14
Teindland seed beds	Profile pit	NJ 295 569	125	Frost cracks present	≈ 20
Clatteringbriggs wood	Profile pit	NJ 290 581	85	38–104 cm + Frost cracks present	12–15
	Test hole	NJ 296 580	85	48–118 cm + Frost cracks present	–
Red burn	Bank section	NJ 302 576	75	below 33 cm Frost cracks present	–
	Gravel pit	NJ 310 552	60	56–180 cm Several frost wedges present	–
Wood of Cairnty	Gravel pit	NJ 307 545	90	Frost wedge present	–
	Gravel pit	NJ 306 518	120	2 m frost wedge present	–
	Gravel pit	NJ 297 510	85	Small frost wedge present	–
	Forest track section	NJ 334 533	165	Basal tips of frost cracks present	12–16
Wood of Cairnty	Roadside cutting	NJ 343 520	125	Frost cracks present in till underlying a solifluction deposit	–
Buried soil section at Teindland	Quarry pit	NJ 297 570	100		7–9

*Altitudes estimated to nearest 5 m by interpolation between contour lines of Ordnance Survey 1:25,000 scale map.

absence from this deposit of a regular pattern of polygonal frost cracks as seen in the red till in nearby exposures along the Red Burn (Table 1) (which would clearly have been just upstream of the Teindland site during any Devensian glaciation) confirms that this 'sandy till' is in fact soliflucted till and lateral moraine derived from the nearby slopes of Teindland Forest. This interpretation is consistent with both the pollen data and particle size

and shape criteria indicative of glacial origin presented by Edwards *et al.* The preferred orientation of pebbles of 110–120° measured by Edwards *et al.* is not in accordance with the orientation to be expected from free downslope flow of a solifluction deposit¹⁰, but it should be pointed out that this measurement was made only a few metres from the position at which the downflow must have stopped at the change of slope. Pebble orientation at this point is, therefore, unlikely to be in the direction appropriate to unconfined free flow, and orientation in concordance with ice flow direction is coincidental.

Climatic data presented by Dansgaard *et al.*¹¹ show that the period 28,000–12,000 b.p. was the most prolonged cold interval during the Devensian stage. The strong indications that the till-like material above the buried organic horizon at Teindland is a solifluction deposit and the probability that the frost cracks in the buried layer are contemporaneous with those developed in red till sections around the site suggest that the age of deposition of the local red till is pre-Devensian, and that Devensian sheet glaciation in this area is improbable.

I thank Mr J. Logan of the Macaulay Institute for the mechanical analyses in Table 1, Dr S. E. Durno of the Macaulay Institute for pollen analyses of the Bogincur farm buried peat and Mr J. Atterson of the Forestry Commission who took the photograph reproduced in Fig. 1.

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Fig. 1 Detail of the largest frost crack present in the 1962 section at Teindland.

An Ediacara-type fauna from South Wales

DURING investigation of the geology of the area south of Carmarthen, South Wales, UK, an isolated small exposure of a finely inter-laminated mudstone and siltstone of possible volcanogenic origin was discovered. This lithology differs from those found hitherto in the Arenig and Old Red Sandstone rocks locally, and does not resemble any of the facies of the recently discovered Tremadoc rocks of the area¹. Samples from this quarry were examined by K. J. Dorning of Sheffield University for microflora, but yielded none. Late last year a few badly preserved disk-shaped impressions, some 1 cm in diameter, were discovered. These were tentatively linked with the trace fossil *Astropolithon* by P. Crimes (Liverpool) and A. Seilacher (Tübingen), who both suggested that if the material proved

to be *Astropolithon* an Early Cambrian age would be indicated.

Further searches were made in the hope of finding material useful for dating. These have now yielded about 50 specimens including some better preserved material which is clearly of medusoid nature. A few of these specimens were subsequently examined by I. P. Crimes and by R. Jenkins (Adelaide), who both confirmed the medusoid nature of the fossils and suggested that they included a form occurring in the late Precambrian Ediacara fauna of South Australia.

Amongst the material so far collected, the genera *Cyclomedusa* Sprigg 1947 (ref. 2) and *Medusinites* Glaessner and Wade 1966 (ref. 3) have been identified. The less well preserved material includes a form resembling the genus *Tribrachidium* Glaessner 1959 (ref. 4). Others cannot at the moment be related to any previously described forms.

The fauna clearly carries elements characteristic of the Ediacara fauna. Late Precambrian soft-bodied organisms are known from various areas of the world⁵ including Charnwood Forest, Leicestershire, UK⁶. In the Carmarthen region, however, until recently the earliest known rocks were of Early Ordovician (Arenig) age. The discovery of Tremadoc rocks there has led to a re-assessment of the age of the underlying rhyolites which are now presumed to be Precambrian. The rocks which have yielded the medusoids appear to rest on the rhyolites, although field relations in such a poorly exposed area are not clear.

No Cambrian rocks (if one excludes the Tremadoc) have hitherto been found in this area, and the possibility that the fauna is of Cambrian age cannot be excluded. It is felt, however, that the absence of any similar fauna in the Cambrian rocks of the St David's area (or elsewhere in Wales), argues against this, as does the apparently complete absence of metazoan skeletal material which would be most unusual for shallow water Cambrian sediments. Glaessner⁵ has given reasons for assigning a late Precambrian age to the Ediacara fauna. On palaeontological as well as regional geological grounds a similar age is suggested for this fauna.

Details of the location of the exposure and a description of the fauna will be published after a full investigation of the site.

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Tachigalia versicolor is a suicidal neotropical tree

WITHIN a year of flowering in *Tachigalia versicolor* Standl. and Wms (Leguminosae-Caesalpinioideae) the leaves drop, the fruit is released and the tree dies. Reproduction occurs synchronously at intervals of several years in this large, much-branched, canopy tree species, but not all of the large individuals in the forest canopy flower and die at once. The species is known from the evergreen and semi-deciduous lowland forests of Panama, south-east Costa Rica and north-west Colombia. Except for a brief mention¹ of the Amazonian *T. myrmecophila*, I have found no previous reference to reproduction followed by death in *Tachigalia* (including *Sclerobium*, an apparently congeneric taxon^{2,3}), nor in any other large, branched, dicotyledonous

Fig. 1 In each case, scale bar equals 10 mm. *a*, *Cyclomedusa* sp.
b, *Medusinites* sp.



tree. My preliminary observations in Peru suggest that this behaviour is characteristic of several of the other 56 species in this genus, most of which occur in the Amazon Basin.

For 10 yr, *T. versicolor* trees have been watched on Barro Colorado Island, Panama, a field station of the Smithsonian Tropical Research Institute in the centre of the Panama isthmus. Initially, nearly 40 individual trees were known and mapped though not tagged. In the past 3 yr a search has revealed 430 trees, and these have been mapped and marked with metal number tags.

From 1967 to 1969, no reproductive individuals were observed. But in 1970 at least 18 large trees flowered. All died within 1 yr, after release of their wind-dispersed fruits. No vegetative regrowth has been observed from the bases of trees or underground parts.

After 1970, no further flowering was seen for 4 yr. In 1974, eighty-five individuals were found in full flower on the island and all were dead by May 1975 (Fig. 1). This represents approximately one-fifth of the 430 known canopy trees. The non-reproductives completely overlap the range of sizes (height and diameter) of the reproductives (Fig. 2). Stem growth-rings are not distinct and the age of individuals at reproduction is not known.

Though the two flowering waves were distinct, the individuals flowering within a given year were not closely synchronised in the initiation of flowering. Some started flowering as early as January (the dry season) and others as late as July (the rainy season). Individual flowering lasts between 3 and 6 months. As a consequence, the flowering period for the whole population lasts almost a full year.

Two trees initiated flowering in 1975. One had its roots naturally grafted to those of an adjacent tree that had flowered



Fig. 2 Trunk of non-reproductive *Tachigalia versicolor* Leguminosae-Caesalpinioideae. Diameter at breast height was 116 cm and height was 35 m. Individuals of both reproductives and non-reproductives are commonly more than 150 cm and 40 m in height.



Fig. 1 View of dying emergent *Tachigalia versicolor* on Barro Colorado Island from Gatun Lake, Panama, February 1975. A few fruits remain on the estimated 7,000 terminal inflorescences. As flowering finishes, the leaves drop off, leaving the canopy crowded with 15-cm fruits that are green and leaf-like. These turn brown and are dispersed by wind in the January–April dry season, as the trunk starts to decay.

in the previous year, and the second was a sickly individual with one branch remaining. No trees were observed to flower in 1976. There were a few partial exceptions to the rule of death following reproduction in 1974: three individuals produced flowers and fruits on only a few of their branches; those branches subsequently died, but the trees as a whole survived.

A suicidal act of reproduction might seem maladaptive for a species of huge trees. These trees do not seem to store nutrients and energy for the one reproductive event, for the quantities of flowers and fruits seem no greater than in comparably sized tree species with repeated reproduction. On the basis of initial observations, I propose that selection has favoured individuals which, by dying and falling, greatly increase the probabilities for their few progeny of survival to maturity in the forest canopy. The parent's death creates a more favourable environment for the development of its offspring, and occurs when the offspring are still abundant—having not yet succumbed to all the sources of juvenile mortality. Because most, if not all, tropical canopy species require an opening or 'light-gap' in the forest canopy for successful maturation^{4,5}, a treefall should strongly augment the opportunities for seedlings in the vicinity of the parent. As with other species that have large wind-dispersed propagules, the seeds and seedlings of *T. versicolor* are rarely found more than 100 m from the parent. Thus most seedlings (when not completely crushed) are likely to be exposed by the fallen parent, or by nearby treefalls resulting from the initial disruption of the forest canopy. *T. versicolor* saplings are often found growing in the openings directly created by the fall of an adult, or in adjacent openings. The rate of growth (or some other important parameter) of the trees I observed could be augmented by the amount of energy and nutrients they do not put into reproduction every year.

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Cross-fertilisation in fucoid seaweeds

EXPERIMENTAL studies on the extent of cross-fertilisation between eggs and sperm of various fucoid seaweeds are few, the results widely divergent and in no case have the experimental conditions been precisely defined. For example, in interspecific crosses between *Fucus serratus* eggs and *Fucus vesiculosus* sperm, values of 0%, 2% and 91% have been recorded (refs 1, 2 and 3 respectively). Hybridisation to a greater or lesser extent on the shore does, however, seem feasible especially since many species are cytologically identical⁴. Indeed, plants showing morphological features intermediate between parental types have been recorded^{5,6}. While some authorities^{7,8} consider that intermediate forms represent no more than ecotypes or phenotypic variants of parental type species, others³ consider that these represent true hybrids, although cytogenetic evidence is lacking. We report here a re-appraisal of the specificity of cross-fertilisation between three fucoid seaweeds. The results show the presence of inherent interspecific and inter-generic barriers to fertilisation. But, interspecific hybridisation may be permitted following the breakdown of these barriers.

Fucoid eggs secrete a polysaccharide cell wall immediately after fertilisation^{10,11}. Accordingly we have used the fluorescent brightener Calcofluor white ST (Cyanamid) to stain zygotes permitting a rapid and direct assessment of the level of fertilisation by ultraviolet microfluorescence (Reichert Diapan microscope fitted with exciter filters BG38 and UG1 and barrier filter GG13+W2B). It has been determined that zygotes which stain positively with Calcofluor subsequently show rhizoid development.

Fertile plants of *Fucus serratus*, *F. vesiculosus* and *Ascophyllum nodosum* were collected from several localities on the west coast of Anglesey, Wales during April 1977, and stored moist for up to 7 d. *Ascophyllum* was included in view of a report⁹ that sperm of this plant can cross-fertilise eggs of *F. vesiculosus* to an extent of 4%. Dioecious species only were chosen for this study since it was considered imperative that any possibility of cross-contamination of gametes should be avoided.

Gametes were released¹⁰ when required and crosses were carried out by incubating 5,000 eggs in concentrations of sperm up to 4,000 per egg, in a total volume of 2 ml for 20 min at 18 °C. Sperm were inactivated by adding 20 µl of 0.2% I₂ in 2% KI and the excess liquid removed from the eggs which had been allowed to settle. The eggs were washed once with seawater and then incubated for 5 min in a saturated solution of Calcofluor in seawater. After removing excess Calcofluor the eggs were washed in seawater once more, then percentage fertilisation was

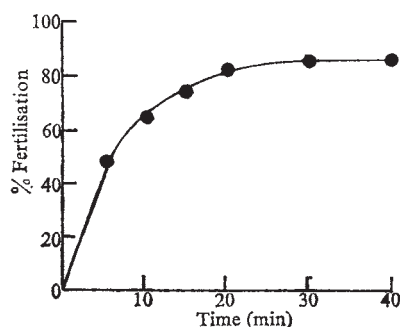


Fig. 1 Time-course of homologous fertilisation in *Fucus serratus*. 5,000 eggs were mixed with 200 sperm per egg.

assessed by counting the number of eggs showing the characteristic fluorescence in a sample of 200 eggs. Control incubations omitted sperm.

Homologous fertilisation in the three seaweeds has similar characteristics with regard to the kinetics of fertilisation and fertilisation efficiency. Fertilisation can be detected within a few minutes of adding sperm but is essentially complete within 20 min (Fig. 1). Fertilisation efficiency is linear at low sperm concentrations, 50% of eggs being fertilised at relative sperm concentrations between 50 and 100 sperm per egg, saturation being approached between 250-500 sperm per egg (Fig. 2a,e,i). Efficiency of homologous fertilisations is independent of the stored age of either male or female plants up to 7 d in the experimental conditions used.

In heterologous fertilisations sperm to egg ratios of up to 4,000 were used to increase the potential for fertilisation. Cross-fertilisation could not be detected with eggs

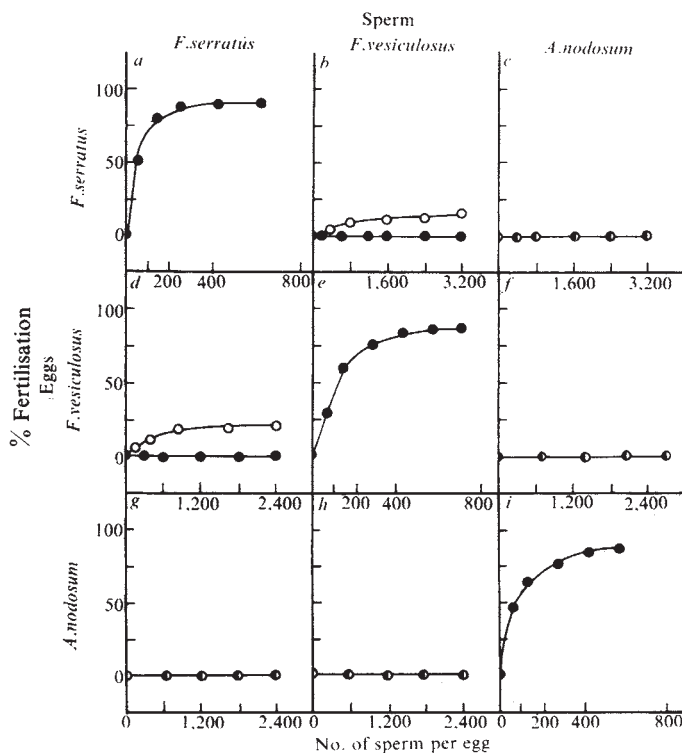


Fig. 2 Fertilisation efficiencies in homologous and heterologous fertilisations. 5,000 eggs were incubated with different concentrations of sperm. ●, Eggs from freshly-collected plants; ○, eggs from 4-5 d stored plants; ◐, eggs from freshly-collected or stored plants.

released from freshly collected plants in any of the six crosses shown in Fig. 2b-d,f-h. But, in the case of *F. vesiculosus* eggs $\times$ *F. serratus* sperm, or the reciprocal cross, low levels of fertilisation were detected up to 20% with eggs freshly released from plants stored for 5-7 d (Fig. 2b,d). Storage time of male plants was immaterial.

The inter-generic crosses between the two *Fucus* spp. and *Ascophyllum* were negative in all conditions of storage and sperm concentrations used. The occurrence of cross-fertilisation between *F. serratus* and *F. vesiculosus* was subsequently confirmed by the occurrence of segmented zygotes in samples incubated for 24 h.

Fucoid seaweeds show a wide range in form in response to environmental factors¹². In addition, hybrids have been described and on the basis of ecological observations and hybridisation experiments it has been concluded that there are no inherent sterility barriers among *Fucus* species and that the barriers which prevent the merging of the different species into one polymorphic complex are ecological rather than physiological, related, for example, to non-coincidence of fruiting periods³ and competition from parental forms². The results of our investigation do not support this conclusion, since direct studies on fertilisation showed that there are inherent barriers to both inter-generic and inter-specific cross-fertilisation. But, the interspecific barriers seem to become less pronounced with age.

Gametes of the two *Fucus* species used in this study are continually differentiated and the older, more mature gametes are periodically released twice per day in the normal tidal cycle. Since stored plants were not subject to this tidal action the population of gametes experimentally released from stored plants will contain a relatively higher proportion of older eggs.

Specificity in seaweed fertilisation, as in sea-urchin¹³ and mammalian¹⁴ fertilisation is probably based on surface-localised complementary macromolecular recognition phenomena. It is tentatively suggested, therefore, that the partial breakdown of an inherent sterility barrier in the interspecific crosses is due to modification in the properties of the appropriate membrane receptors of the older egg cells. The molecular nature of these receptors is being investigated now. But, until more is known about the effects of external factors on the biochemistry of gamete development and the resulting implications on fertilisation, speculation on the ecological significance of the reduced specificity would be premature.

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Hedgehogs use toad venom in their own defence

I REPORT here that toxic secretions evolved in prey organisms such as toads (*Bufo*) as chemical anti-predator mechanisms are used by hedgehogs (Insectivora, Erinaceidae) to enhance their own mechanical anti-predator adaptations—the spines, modified hairs, of the back. The secretions are taken into the mouth and licked on to the spines. In spite of interest in chemical, mechanical and behavioural anti-predator adaptations of both invertebrates and vertebrates¹ there have been no reports of the behavioural utilisation of the anti-predator secretions of one species by another for its own defence. Toad skin is one of many irritating substances used by hedgehogs in self-anointing, and the presence of any of these, fresh or dried, on the spines would probably increase the pain or potential of infection to the would-be predator.

The survival value of spines is enhanced by the hedgehog rolling into a ball or 'boxing' when contacted or approached by a predator. Boxing consists of erecting the spines over the head and lunging into the approaching predator. Hedgehogs also display a curious behaviour pattern known as *Selbstbespeicheln* or self-anointing. Published reports²⁻⁴ and my observations on 18 *Atelerix pruneri* and one *Hemiechinus auritus* agree that this behaviour involves frothing at the mouth then spreading the froth with the tongue on to the spines. Many possible functions of this behaviour have been considered and rejected².

Substances which naturally release self-anointing behaviour are either novel or noxious (irritating to mucosal membranes). Other common releasers are tobacco, soap and salamander skin secretions. Toad skin has often been mentioned as a releaser. Because toads (*Bufo*) occur throughout the natural range of hedgehogs, are commonly eaten by hedgehogs⁵ and produce toxins in the granular glands of the skin it is possible that hedgehogs use *Bufo* skin toxin in their own defence.

Each time a toad (50+ times) with skin intact was contacted by adult or laboratory-reared juvenile hedgehogs they displayed self-anointing behaviour. When the following species of *Bufo* were offered to hedgehogs, the results were identical: *B. alvarius*, *B. americanus*, *B. boreas*, *B. marinus*, *B. quercicus*, *B. regularis*, *B. terrestris* and *B. woodhousei*. Toad skin, fresh (six trials) or dried (six trials), or excised toad paratoid glands (15 trials) always elicited self-anointing, but toads with the skin removed (six trials) were always eaten without self-anointing. Bullfrogs, *Rana catesbeiana*, and white mice (at least 30 each) were eaten without self-anointing. When hedgehogs attack toads they first bite and chew the paratoid glands (these contain the greatest concentration of skin toxins) then distribute the resultant frothy mixture of toad skin secretion and saliva over the spines (Fig. 1); the toad is then often eaten. In two cases *Hemiechinus* grasped the toad in its mouth and rubbed the skin over its spines (Fig. 2).

Observations of six *Atelerix* born and raised in captivity indicate that the self-anointing (Fig. 3) and other anti-predator behaviour are expressed before the eyes open at age 18-24 d. Long white spines are emergent within minutes after birth and a second stiffer set of dark spines emerges within 2-4 d. From 6 to 10 d after birth the young begin boxing behaviour, orienting the body towards any contact, and hissing. They can roll into a full ball by 2 weeks. Self-anointing is fully developed at 15-16 d (13-d-old juveniles do not exhibit this behaviour) in response to various stimuli such as sour milk, hands freshly washed with soap, or tobacco juice. These juveniles also licked substances from their mother's spines and applied them to their own spines.



Fig. 1 Adult *Atelerix* self-annointing after chewing a toad (*Bufo*). The forth of saliva and toad skin secretion is being applied to the spines with the tongue.

To determine whether self-annointing with toad skin secretion would increase the pain inflicted by penetration of a hedgehog spine, spines were removed from an *Atelerix* and jabbed into the inner forearm of the author and six volunteers. Each subject jabbed himself with four spines in random order (only the author knew the order). The spines were treated as follows: (1) spines washed in alcohol; (2) spines washed then coated with hedgehog saliva; (3) spines washed then coated directly with *Bufo marinus* skin secretion; (4) spines freshly self-annointed with *Bufo marinus* skin secretion. In only one of seven cases did spines of groups (1) or (2) cause any reddening, and there was no pain or burning other than the mechanical pain of puncture. In group (3) six of seven subjects and in group (4) all subjects experienced immediate intense local burning. Splotchy red areas 8–46 mm ($\bar{x}$ = 24.6 mm) developed around the puncture and reached their greatest development in 10–40 min ($\bar{x}$ = 18.1 min). Burning lasted for 5–60 min and varied greatly in intensity. The reaction to two self-annointed spines dried for 1 week was the same as to freshly self-annointed spines.

Self-annointing behaviour is not only widespread among hedgehogs, having been reported in *Erinaceus*, *Aethechinus*, *Paraechinus*, *Hemiechinus* and *Atelerix*², but has also been described in the hedgehog tenrec *Echinops*³, an insectivore

Fig. 2 *Hemiechinus* rubbing *Bufo* against spines. Froth is visible on spines along the side and between the ears.



of the family Tenrecidae convergent with the true hedgehogs in the presence of spines. Tenrecs, in contrast to hedgehogs, wipe the mixture of stimulating substance and saliva over the spines with the forepaws. In hedgehogs the mixture is always licked directly on to the spines, never on to the forepaws. These stereotyped behaviour patterns of both hedgehogs and tenrecs are released by chemical stimuli perceived by taste or smell, involve increased salivation, and

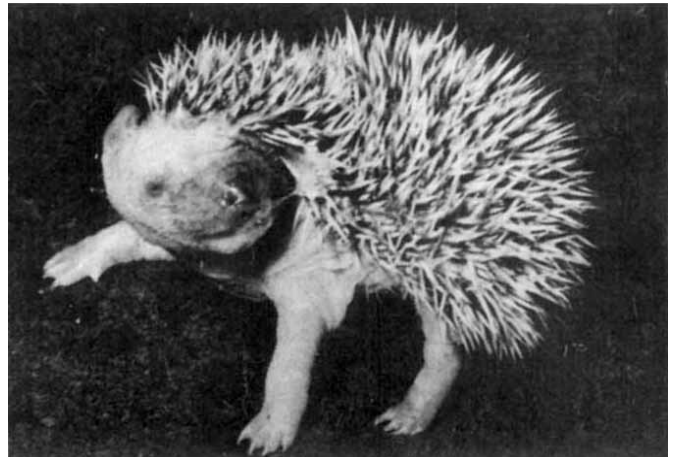


Fig. 3 Seventeen-day-old juvenile *Atelerix* frothing at the mouth and self-annointing after licking tobacco juice. The eyes have not yet opened.

include the spreading of this saliva–substance mixture on to the spines. The behavioural components of self-annointing in the two families of insectivores are quite different and were apparently developed independently⁴. The presence of these different behaviour patterns which both utilise saliva as a vehicle for the application of irritating substances on to spines suggests that these behaviours increase fitness. The fact that the anti-predator behaviour patterns including self-annointing are innate and fully developed in hedgehogs before leaving the nest further supports their importance.

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Diets of early Miocene African hominoids

MOST reviewers of East African early Miocene apes have been impressed by their similarity, as an adaptive array, to living Cebidae. These apes are most frequently pictured as small to medium-sized cebid-like quadrupeds occupying arboreal^{1–6}, fruit- and leaf-eating^{3,7,8}, tropical rainforest⁹ niches. It has also been suggested that the decline in the diversity of apes in middle to late Miocene times was triggered by ecological competition from rapidly radiating cercopithecids^{9–11}. In this report

I examine the molar structure of early Miocene hominoids to see whether or not these species occupied a wide spectrum of fruit- and leaf-eating niches, similar to those of today's cercopithecids.

I have analysed 65 specimens of extant pongids and hylobatids from the Musée Royal de l'Afrique Centrale, the American Museum of Natural History and the US National Museum of Natural History. The sample includes 10 of the 11 recognised ape species¹². The early Miocene fossil apes come from the collections of the Kenya National Museum and the British Museum of Natural History. Three species of New World monkeys were also examined. Twelve previously described dimensions¹³ of unworn lower second molars were estimated: The mesiodistal M_2 length, used as an estimate of overall size; the heights of the metaconid and hypoconid; the lengths of shearing blades 1–8; and the summed surface areas of the trigonid and talonid basins. Cusp heights are interpreted as reflecting capacity for resistance to dental wear; shearing blade lengths and total basin areas reflect the relative importance of shearing, crushing and grinding in mastication. To eliminate the effects of allometry on these variables, I selected different-sized species with diets composed primarily of fruit (two *Pan* and five *Hylobates* species). For this group, 11 regression lines for the $\log_e$ of the molar dimensions (with $\log_e$ length as the independent variable) were fitted by the least squares method. The distance each species' molar dimensions falls above or below the *Pan-Hylobates* regression was expressed as a percentage of the expected dimensions for that tooth length¹⁴. The percentage values derived from each of the 11 regressions were used to produce a correlation matrix from which principal components were extracted. The resulting factor loadings (Table 1) were used to produce principal-component values for each specimen studied. The mean principal-component value for each species is shown in Fig. 1. Student's *t* tests were used to assess the significance of differences between the species means on each component axis. The factor loadings derived from this

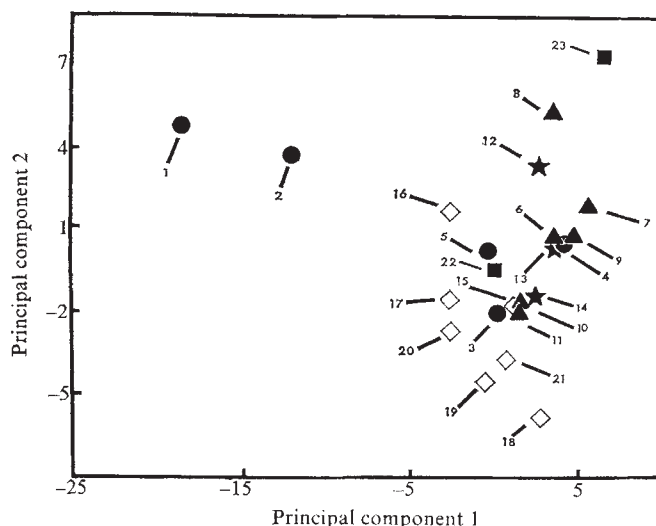


Fig. 1 Plot of principal components scores of primate M_2 s along the first two principal components axes. Sample sizes are included in parentheses. Extant Pongidae (●): 1, *Gorilla gorilla beringei* (8); 2, *Gorilla gorilla gorilla* (6); 3, *Pan troglodytes* (10); 4, *Pan paniscus* (11); 5, *Pongo pygmaeus* (5). Extant Hylobatidae (▲): 6, *Hylobates lar* (5); 7, *Hylobates hoolock* (2); 8, *Symphalangus syndactylus* (3); 9, *Hylobates moloch* (5); 10, *Hylobates klossi* (5); 11, *H. agilis*. Cebidae (★): 12, *Alouatta seniculus* (5); 13, *Ateles belzebuth* (5); 14, *Cebus capucinus* (5). Extinct Hominoidea (◇): 15–17 Songhor; 15, '*Limnopithecus*' *legetet* (3); 16, *Dryopithecus gordonii* (6); 17, *Dryopithecus maor* (3). 18–21, Rusinga Island; 18, '*Limnopithecus*' *legetet* (2); 19, *Dryopithecus africanus* (5); 20, *Dryopithecus nyanzae* (7); 21, '*L.*' *macinnesi* (8). Cercopithecidae (■): 22, *Cercocebus albigena* (6); 23, *Colobus badius* (6).

Table 1 Factor loadings for the first two principal components of the analysis of Z-score corrected, % difference data of the lower second molars of 11 extant apes

Variable	Factor loadings	
	Component 1 (50% of variance)	Component 2 (17.7% of variance)
M_2 hypoconid height	0.878	-0.034
M_2 metaconid height	0.596	0.339
M_2 shearing blade 1	0.363	0.484
2	-0.891	0.102
3	0.277	0.703
4	-0.674	0.500
5	0.767	0.438
6	0.587	-0.587
7n	-0.759	0.494
8	-0.780	-0.052
Total crushing area (9,10 n, x)	0.940	0.248

analysis also were used to produce principal-components values for specimens of living ceboids, Old World monkeys and fossil hominoids. The species means for these forms are shown in Fig. 1. In another principal components analysis, all living and fossil species were run together. The resultant factor loadings and principal-component values were extremely similar and are not reported.

The primarily folivorous species, *Gorilla*¹⁵ and *Symphalangus*¹⁶ are clearly separated morphologically from other, more frugivorous, pongid and hylobatid species^{17–9} on the first and second principal component axes which account for 67.7% of the total variance of the system (Fig. 1). The first principal component separates *Gorilla gorilla* from all other species by a combination of high cusps, enlarged crushing basins and elongate molar crests 5 and 6 (Table 1). But, the first principal component

does not separate folivorous from frugivorous hylobatids. The second principal component separates folivorous from frugivorous species. The former have relatively high metaconids and a great overall amount of shearing and crushing (Table 1). The mean second principal-component values of *Gorilla gorilla* subspecies and *Symphalangus syndactylus* are significantly larger (at the 95% confidence level) than those of all other pongid and hylobatid species tested. This result conforms with the diet-molar morphology correlation seen in other mammalian groups. Folivorous species of Old World monkeys and phalangeroid marsupials also have better developed shearing blades than frugivorous species²⁰. Leaves, bark, buds and other folivorous materials tend to contain large amounts of structural carbohydrates (cellulose, hemicellulose) which require thorough trituration facilitated by well developed molar structures, to enhance digestibility²⁰. In contrast, fruits tend to be lower in structural carbohydrates and higher in soluble carbohydrates which are highly digestible even without full trituration²¹.

Before using this principal components analysis as a model for comparisons of Miocene species, its reliability as a predictor of dietary habits was tested for representative folivorous and frugivorous Old and New World monkeys. To do this, Z-standardised percentage scores were tabulated from the *Pan-Hylobates* regressions for the highly frugivorous New World species *Ateles* and *Cebus*²² and the folivore *Alouatta*^{22,23}. These species' values are interpolated into Fig. 1. *Ateles* and *Cebus* fall with the frugivorous hylobatids and pongids on the second principal component and have significantly smaller (at the 95% confidence level) second-component values than do the folivorous hominoids. The second principal-component value for *Alouatta* is significantly larger than all frugivorous ceboids and hominoids analysed, and is not significantly different from the more folivorous *Symphalangus* or *Gorilla* species. Apparently, the morphological distinctions between fruit- and leaf-eating cebids are analogous to those seen between fruit- and leaf-eating hominoids. Also interpolated into Fig. 1 are the principal-

component values for two species of cercopithecoid monkey, *Cercocebus albigena* and *Colobus badius*. The former is primarily a fruit eater whereas the latter eats primarily leaves. *Cercocebus* falls with primarily fruit-eating hominoids and cebids, whereas *Colobus* falls with the leaf-eating species of these groups. It may be concluded from the above that second lower molar morphology can be used to reliably separate extant fruit-eating from leaf-eating Higher Primates.

Lower second molars of fossil apes from two East African Miocene sites, Songhor and Rusinga Island, spanning the time period of 18–20 myr BP (ref. 24) were measured. The material included specimens of six of the seven species known from these sites⁹: *Dryopithecus gordonii*, *D. major*, *D. nyanzae*, *D. africanus*, *'Linnopithecus' macinnesi* and *'L.' legetet*. The lower dentition of *D. vancouveringi* is not yet known (identified). As with the Old and New World monkeys reported above, Z-standardised percentage scores were calculated from the *Pan-Hylobates* regressions, principal-component values were calculated for individual specimens and the species, mean was determined for each species grouped by locality. The species' values are plotted in Fig. 1. The Songhor specimens of *D. major*, and *'L.' legetet* fall with living frugivorous hylobatids on the second principal component, and are very close to *Pan troglodytes* as well. Both the fossil species have significantly smaller second-component values than extant folivorous species at the 95% confidence level. A frugivorous diet for these species is indicated. *Dryopithecus gordonii* falls between the ranges of living frugivores and folivores on the second principal component axis. This species is not significantly different from either *Gorilla gorilla gorilla* or from several of the species of *Hylobates*. Rusinga Island specimens of *D. nyanzae*, *D. africanus*, *'L.' legetet* and *'L.' macinnesi* fall with frugivorous extant hominoids and have significantly smaller second-component values than extant folivorous species. Points 15 and 18 in Fig. 1 representing *'L.' legetet* from two different sites are not significantly different from one another at the 95% confidence level. This analysis strongly indicates that the known early Miocene hominoids (with the possible exception of *D. gordonii*) probably had diets consisting largely of fruit. In this respect they show less dietary diversity than the pongids of hylobatids of today, since members of both those groups have folivorous representatives. Judging from the molar morphology *D. major* has no apparent dietary affinity with *Gorilla*, contrary to previous suggestions⁸.

Early Miocene apes apparently were quite similar, adaptively, to some extant cercopithecines and a case can be made that monkeys replaced apes in middle to late Miocene times. Early apes have been collected at sites which sample tropical rain-forest environments⁹ where cercopithecines are abundant today, in terms of species numbers. Early apes had far greater species diversity than the apes of today and in this respect they mirror the diversity of living forest-dwelling cercopithecines⁹. Dental dimensions suggest that early apes and living forest dwelling cercopithecines have a similar body size range (2–40 kg). Most of the early Miocene apes are similar to extant cercopithecines in being arboreal quadrupeds^{1–6} with some of the larger forms being at least partially terrestrial⁷. Finally, early Miocene apes were primarily frugivorous, as is the case with many arboreal cercopithecines of today.

But these similarities should not be taken as demonstrating an identity in the adaptive zones of early Miocene apes and extant forest monkeys. There is no evidence that any early Miocene hominoids fed on seasonally high proportions of leaves, bark, buds, or grasses, as do some cercopithecines today. There were no colobine vicars among the early Miocene hominoids. Thus, in addition to displacing early hominoids, the cercopithecoid adaptive radiation may also have come at the expense of other arboreal and semiterrestrial species. Possibly the African colobine leaf-eating radiation displaced some hyracoids, anomalurid rodents or arboreal reptiles, some of which eat bark and leaves^{25,26}.

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Further study of an auditory illusion

DEUTSCH¹ reported an interesting auditory illusion when different tones are presented to the two ears. She used chords alternating every 250 ms between 400 Hz to the left ear, 800 Hz to the right and 800 Hz to the left, 400 Hz to the right. Subjects never heard the correct pattern; they perceived most often a low tone in one ear alternating with a high tone in the other. Right-handed subjects tended to hear the high tone in the right ear and the low tone in the left ear, but left-handed subjects showed no such tendency. Deutsch and Roll later found that right-handed subjects tended to report the sequence delivered to the right ear rather than that to the left, but the tone reported tended to be localised in the ear receiving the higher frequency². They concluded that there were separate mechanisms for perceiving location and pitch, and that the right ear preference for pitch was related to cerebral dominance. Efron and Yund^{3,4} reported a related phenomenon using a sequence of two pairs of tones: 1,500 Hz to the right ear, 1,900 Hz to the left followed by 1,900 Hz to the right ear, 1,500 Hz to the left. Again most subjects heard a single note each time. Efron and Yund systematically varied the relative intensity of the two tones and found that the location of the perceived sound depended critically on this variation, always being heard towards the side receiving the more intense sound. If the two intensities were exactly balanced for an individual, then the perceived sound was subjectively located in the midline. The subjective pitch of the sound heard was a separate function from its location, and frequently the pitch of the less intense sound was heard, but localised in the ear receiving the more intense sound. We have used Deutsch's frequencies of 400 Hz and 800 Hz, and also 457 Hz and 857 Hz. We presented two consecutive pairs of tones, systematically varying the intensity, and confirmed Efron and Yund's results. Using equal intensities we obtained different results from Deutsch.

The tones lasted 250 ms, with a rise and fall time of about 10 ms, and were presented at an intensity of 65 decibels $2 \times 10^{-3} \text{ N m}^{-2}$ through TDH-39 earphones.

Twenty-four right-handed subjects, without extensive musical training, were tested with an audiometer at the two frequencies used and any small difference in acuity was approximately balanced by adjusting the intensity of the tones. Subjects were first given each tone separately as standards of high and low, and then were allowed to listen to the pair of chords as often as they wished by pushing a button to obtain a presentation. For each pair of chords they were asked to indicate on a response sheet exactly what they heard in terms of pitch and location. Each subject received eight stimulus configurations; on two of these the tone of higher frequency was attenuated by 10 dB and on another two the lower tone was attenuated by 10 dB.

A second experiment on another 24 subjects was identical, except that frequencies of 457 Hz and 857 Hz were used, to see if there was any difference with tones lacking a simple harmonic relation.

The results relating to the relative intensity of the two tones are clear. In both experiments, when one of the tones was attenuated by 10 dB, then if only one tone was perceived it was located in the ear receiving the higher intensity on 85% of cases. The pitch of the tone heard was not necessarily that of the more intense tone. This confirms the results of Efron and Yund, that the location of the perceived sound depends on the relative effective intensity of the tones for each individual subject. It also suggests that Deutsch's finding that the high tone tends to be localised in the right ear and the low tone in the left may be due to small intensity differences, or to differences in acuity between the ears of individual subjects.

What subjects hear, as opposed to where they hear it, is a more complex function. Table 1 shows a summary of the results when the two tones are of approximately equal intensity. For each chord (High-L, Low-R or High-R, Low-L) is shown the number of times it was heard as a chord, or single high, low or medium note, irrespective of location. Each of the twenty-four subjects made four observations of each chord. Order effects were not significant.

Table 1 Number of times each chord was heard as a chord, or single high, low or medium pitched note

Stimulus frequencies	400 Hz/800 Hz		457 Hz/857 Hz	
	High-L	High-R	High-L	High-R
Stimulus chord	Low-R	Low-L	Low-R	Low-L
Heard as:				
Chord	0	0	30	33
High	42	40	40	27
Medium	22	28	9	3
Low	32	28	17	33

L, left; R, right.

The perception of a single note is very clear using frequencies of 400 Hz and 800 Hz; a single note was heard on all occasions. With frequencies of 457 Hz and 857 Hz a single note was reported about 66% of the time—but nearly half the subjects heard a chord on some occasion. The illusion seems very strong if the notes have a simple harmonic relation and less strong, but still common, if they do not.

No overall pattern emerges from the detail of what is heard when only one note is reported, but Deutsch's result that the right ear determines what is heard is not confirmed. Whether the notes have a simple harmonic relationship or not, the stimulus configuration does not significantly influence the pitch of the reported note. The function determining what is heard is complex, and shows consider-

able individual differences. Individual subjects tend to be fairly consistent in their preferences.

The illusion falls into the class of 'curious binaural phenomena' reviewed by Tobias⁵, who concluded that 'any similarity between two sounds may lead to their fusion, if the similar factors fall below 1,500 Hz and come close to coinciding in time'.

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Racial differences in the frequency of Q and C chromosomal heteromorphisms

THE biological and clinical implications of human chromosome heteromorphisms are poorly understood. These heteromorphisms are limited to certain groups, and are heritable¹. Consistent variability between people, in both size and staining properties, is observed. This may reflect either structural or biochemical variations. Before chromosome banding procedures, the length of the Y was known to vary from person to person and from one ethnic group to another². Some autosomal regions were also found to differ in various racial groups^{3,4}. Using Q- and C-banding procedures, we have regions scored were (1) on the long arms (*qh*) of chromosomes 1, 9 and 16; (2) on the short arms (*p*) of the acrocentric autosomes (13–15 and 21–22); and (3) on the centromere (*c*) of all other chromosomes. The results indicate significant differences between the samples of black and white children.

Chromosome studies of peripheral blood lymphocytes were originally done on 4,342, 7–8-yr-old, children from the Collaborative Perinatal Study through the collaboration of cytogenetic laboratories in five cities. The culture and harvest methods are described elsewhere⁵. Most of the data presented here, however, were obtained from a subsample of 415 children. This was derived, using random numbers, from the larger study group and designed to give equal numbers of children of each race above and below an IQ of 85 (ref. 6). Slides from these children were sent to the coordinating centre for Q-banding. The same slides from 200 of the 415 children were also successfully C-banded. In all cases, 5–10 cells were photographed and analysed; two karyotypes were prepared. If C-banding was done, two dual karyotypes with Q- and C-banded chromosomes were prepared from the same cells. It is important to note that the C-banding of all chromosomes could be studied because each chromosome was preidentified by Q-banding. Without this sequential staining, only 1, 9, 16 and Y can be studied.

Heteromorphisms were scored according to predetermined criteria without knowledge of race, IQ or other parameters. For Q-banding, the five intensity levels established in 1971 at the Paris Conference were used⁷. The levels are brilliant, intense, medium, pale and negative; for convenience, they are referred to as levels 5–1, respectively. For C-band heteromorphisms, the karyotype was arranged by decreasing size of the C-band region from largest to smallest⁸. This permitted comparison of C-band regions on homologous and non-homologous chromosomes having similar sized heteromorphisms. Classification of both Q- and C-band heteromorphisms was done by two independent observers, whose scores were virtually identical.

There were 190 black and 194 white children: 98 of the black and 52 of the white children had an IQ less than 85 and the remaining children (92 black and 142 white) had an IQ of 85 or above.

Before scoring the heteromorphisms, each set of slides was scored for quality of culture and quality of banding. There were no significant differences in these distributions between races or between IQ groups. In all groups, the number of heteromorphisms detected directly reflected the quality of the culture. On the average 3.3 Q- and 3.9 C-band heteromorphisms per child were found.

The frequencies of Q-band heteromorphisms in the two racial groups are given in Table 1. An average of seven heteromorphisms was present in black children and an average of five in white. Where there were significant racial Q-band differences, blacks tended to brilliant or intense heteromorphisms on chromosomes 3, 13 and 22. Chromosome 4 was the single exception. Even when the difference was not statistically significant, the frequency of level 4 and 5 heteromorphism tended to be greater in blacks. We conclude that bright Q-band heteromorphisms are generally more common in blacks.

The centromere (C-band) heteromorphisms were studied in 95 white and 97 black children. These were much less frequent than the prominent Q-band heteromorphisms or *qh* heteromorphisms in 1, 9 and 16. Therefore, data for all chromosomes were pooled. In the white children, the number with small

Table 1 Frequencies of Q-banding heteromorphisms in white and black ethnic groups

Chromosome region	Level (s)	White (N = 205)		Black (N = 210)		P value
		N	Prop.*	N	Prop.*	
3c	4	57	0.28	89	0.42	<0.01
	5	119	0.58	119	0.57	ns†
4c	4,5	42	0.20	20	0.09	<0.01
	5	87	0.42	113	0.54	<0.01
13p	4	22	0.11	70	0.33	<0.001
	5	9	0.04	20	0.10	<0.01
13s	4,5	23	0.11	27	0.13	ns
14s	4,5	2	0.01	6	0.03	ns
15p	4,5	21	0.10	21	0.10	ns
21p	4,5	1	0.005	4	0.02	ns
21s	4,5	16	0.08	28	0.13	ns
22p	4,5	4	0.02	8	0.04	ns
22s	4,5	12	0.06	29	0.14	<0.01

*Proportion of people, either heterozygous or homozygous. Significantly higher frequencies are underlined.

†ns, not significant χ^2 value.

(N = 44) and large (N = 44) C-band regions was equal, whereas 50 black children had small and 78 had large heteromorphic regions. Overall, larger than usual C-band regions were clearly more common in blacks ($P < 0.005$). Most of this difference was due to chromosomes 4, 18 and 19. No individuals in the white sample had prominent, large centromere heteromorphic regions for chromosomes 4, 18 and 19, but 7–9% of the black children had large centromere regions for these chromosomes. Five white children had a large centromeric C region in an X chromosome in contrast to only one black child.

The prominent C-band regions on chromosomes 1, 9 and 16 were also studied. These heterochromatic (*h*) regions are usually on the long (*q*) arm of these chromosomes just below the centromere and are termed *qh* regions. There were no obvious racial differences in either extremely small or large *h* regions for chromosomes 1, 9 or 16. Because of greater complexity of these data, they were analysed on a per chromosome basis, rather than a per person basis and will be reported separately by us. Occasionally, however, they seem to be inverted and to be partially or wholly on the short arm of these chromosomes. Although there is no information which permits us to determine whether these are true inversions or whether they represent

Table 2 Frequencies of 1 and 9 *q* inversions* in white and black ethnic groups in the overall sample

Inversions	White (N = 3084)		Black (N = 1780)		P value
	N	%	N	%	
1 <i>h</i> partial	14	0.45	1	0.06	<0.05
9 <i>h</i> partial	17	0.55	8	0.45	ns
9 <i>h</i> complete	4	0.13	19	1.07	<0.001

Partial inversions were defined as those in which the centromere was in the middle of the *h* region and 'complete inversions' of chromosome 9 as those in which there was a metacentric 9 with the entire *h* region being in the short arm.

unusually placed centromeres, the term 'inversion' has been used for a decade and remains reasonably descriptive. Partial inversions were scored when there was a dumbbell-shaped C-region, with the centromeric constriction near the centre of the dark-staining C-band regions. Complete inversions were those in which all of the C-band positive material was found on the short arm. These heteromorphisms were analysed in the overall sample of 4,342 children since they can be reasonably well detected in G-banded and unbanded cells (Table 2).

Complete inversion of the 9*qh* region was much (six times) more common in blacks, as had been reported before banding⁴. Conversely, partial inversions of chromosome 1 were much more common (eight times) in whites. No complete inversions of chromosome 1 and no inversions, even partial, of the C-band region on chromosome 16 were observed in our sample, suggesting that these inversions are uncommon in both racial groups.

Previously published population surveys using banding techniques have dealt solely with white populations^{8–13}. Only in the present survey, therefore, can the possibility of racial differences be studied. There was clearly a significant black/white difference in the frequency of Q- and C-band chromosome heteromorphisms, with blacks generally having more prominent heteromorphisms than whites. Presumably this reflects a greater degree of redundancy of certain classes of human satellite DNA in these regions. Studies with annealing techniques have not yet been carried out in individuals of different racial or ethnic backgrounds. As with ethnic differences in blood groups, enzyme types and other single gene markers, the evolutionary significance of these frequency differences is purely conjectural. They are clearly of similar genetic and anthropological interest, however, and may be of value in planning linkage and population research.

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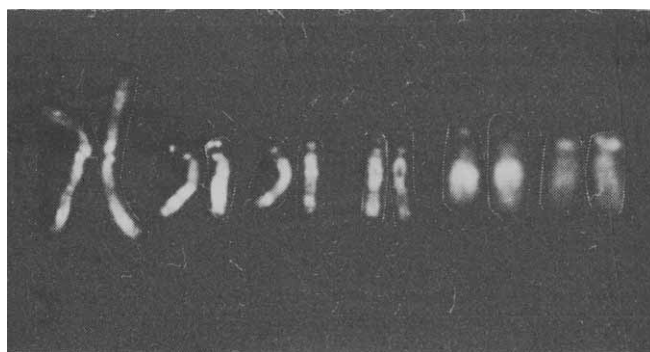


Fig. 1 Q-banded direct chromosome preparation from case 2. All the homologous chromosomes are homozygous for banding polymorphisms.

Androgenetic origin of hydatidiform mole

CLASSIC hydatidiform mole is the product of an abnormal pregnancy with grossly swollen chorionic villi, but without an embryo, cord or amniotic membrane. Histologically, the villi are characterised by advanced hyperplasia and anaplasia of the trophoblast, oedema of the stroma and the absence of foetal capillaries¹. A malignant change to invasive mole or choriocarcinoma is frequent. The karyotype of the mole is predominantly, if not exclusively, 46,XX. We have made a cytogenetic study of the origin of hydatidiform mole and found that it receives only paternal chromosomes and is therefore androgenetic in origin.

Karyotyping was done on 20 moles: in 13 cases on uncultured villi, in three cases on cultured villi and in the other four cases on both cultured and uncultured villi. Ten moles were of Caucasian origin and ten were of Japanese origin. All had a 46,XX karyotype.

The mode of inheritance of molar chromosomes was studied using fluorescence Q and R-band polymorphisms as markers, as described before². Seven moles, two Spanish and five Japanese in origin, and the parents were studied (Table 1). Chromosome slide preparations were made from both cultured and uncultured villi of molar specimens, and from short term lymphocyte cultures of the parents. Seven pairs of chromosomes (nos 3, 4, 13, 14, 15, 21 and 22) from each of the seven moles were scored for fluorescence banding polymorphisms (Table 1). In all of the 49 pairs scored, homologous chromosomes were homozygous for banding polymorphisms (Fig. 1). In 26 pairs of chromosomes, neither of the maternal homologues was transmitted to the mole. In 20 of the 26 pairs, a paternal chromosome could be shown to be inherited in duplicate by the mole (Fig. 2). Comparison of the paternal and molar chromosomes revealed that in 30 pairs the paternal homologues were

heterozygous for banding polymorphisms, while those of the moles were homozygous (Table 2).

Stromal cells in villi were studied for the X chromatin in 47 moles, 18 Caucasian and 29 Japanese in origin. All but three were X-chromatin positive, varying in frequency between 73% and 85.5%. Of the three X-chromatin negative moles, two were Y-chromatin negative, while the remaining one was Y-chromatin positive in 54% of its stromal cells. Necrotic changes could be excluded as a cause for the latter findings. Karyotyping was not successful in the three X-chromatin negative moles.

A total of 35 couples each with a molar pregnancy were karyotyped, and two of the women had a balanced reciprocal translocation: t(7;8)(q22;p21) and t(3;22)(p11;p11). The mole from the first translocation carrier woman revealed a 46,XX karyotype, while that from the latter failed to yield a karyotype.

Our results indicate that moles are androgenetic in origin. Androgenesis is the development of an ovum under the influence of a spermatozoan nucleus, the egg nucleus being either absent or inactivated³. Androgenetic 'zygoids' are usually haploid. Diploid androgenesis, as in these moles, involves a male chromosome complement that has become diploid through fertilisation by two sperm (dispermy), by a diploid sperm or by a haploid sperm followed by duplication of its chromosomes. In the case of dispermy, however, there would be an equal number of heterozygous and homozygous chromosome pairs. The fact that all 30 informative chromosome pairs in the moles studied were homozygous essentially rules out this possibility. The chromosome findings are compatible with either fertilisation by a diploid sperm due to nondivision at second meiotic division or fertilisation by a haploid sperm followed by duplication of its chromosomes and nondivision at first 'blastomere' mitosis. These two possibilities are indistinguishable by the method we used. Both these events would produce a diploid zygoid containing either XX or YY sex chromosomes. Because

Table 1 Mode of inheritance of polymorphic chromosomes in hydatidiform moles

Case	Ethnic group	Chromosome preparation	Karyotype	3	4	13	14	15	21	22	Total no. P	patII
(1)	Spanish	Direct	46,XX	patII			patII	pat	patII		4	3
(2)	Spanish	Direct	46,XX						patII	patII	2	2
		Cultured										
(3)	Japanese	Direct	46,XX			pat	patII		patII	patII	4	3
		Cultured										
(4)	Japanese	Direct	46,XX	pat		patII	patII	patII			4	3
		Cultured										
(5)	Japanese	Cultured	46,XX		patII	patII	patII	pat	patII		5	4
(6)	Japanese	Cultured	46,XX			patII	patII	patII			3	3
(7)	Japanese	Direct	46,XX			pat		patII	pat	patII	4	2
		Cultured										

Direct, direct preparation. Karyotyping was done without intervening culture period, in order to avoid possible outgrowth of maternal decidual cells. pat, Paternal in origin, ascertained through the exclusion of inheritance of maternal homologues. patII, Paternal second meiosis, ascertained through the transmission of a paternal chromosome in duplicate to the mole.

Table 2 Comparison of fluorescence-banding polymorphisms in paternal and molar chromosomes

Case	Tissue	Polymorphisms involving chromosomes numbers							Total no.	
		3	4	13	14	15	21	22	Heterozygous	Homozygous
(1)	P	ab			ab		ab	ab	4	
	HM	aa			aa		aa	aa		4
(2)	P	ab					ab	ab	3	
	HM	aa					aa	aa		3
(3)	P			ab	ab	ab	ab	ab	5	
	HM			aa	aa	aa	aa	aa		5
(4)	P			ab	ab	ab	ab	ab	5	
	HM			aa	aa	aa	aa	aa		5
(5)	P	ab	ab	ab	ab		ab		5	
	HM	aa	aa	aa	aa		aa			5
(6)	P	ab		ab	ab	ab		ab	5	
	HM	aa		aa	aa	aa		aa		5
(7)	P				ab	ab		ab	3	
	HM				aa	aa		aa		3
Total no:		4	1	4	6	4	5	6	30	30

P, paternal; HM, hydatidiform mole; a and b do not represent two specific phenotypes, they are merely symbols.

a cell must have at least one X chromosome to survive, presumably only the XX 'zygoids' could survive to become moles.

The X-chromatin negative moles in our study may have had two X chromosomes with neither X inactivated. Alternatively, they may have arisen through a mechanism different from that

in the seven moles of which the chromosome source has been studied. Abnormal first meiotic division in the male, for example, would result in XY diploid sperm, which in turn could produce XY moles. The X-chromatin negative, Y-chromatin positive mole in this study may have arisen by this mechanism.

The finding of two translocation carrier women among the 35 couples with molar pregnancies is interesting. Their frequency of 6% among the women studied is higher than that of 0.3% in the general adult population⁴. The presence of translocation chromosomes may interfere with female meiosis and encourage the production of ova with absent or inactivated nuclei.

The high malignancy rate in hydatidiform mole could be explained by a recessive mutation of a gene (or genes) which controls cell growth. Because the homologous chromosomes in the mole are genetically homozygous except for the genes exchanged through crossover, they are also homozygous for the mutation and thus may escape from normal growth control.

Wide geographic variations have been known in the frequency of moles, which are more frequent in Asian countries⁵ as well as Latin American countries of the tropical zone⁶ than European and North American countries. The results reported here indicate that the Asian disease is the same as that found among the Caucasians. This finding, however, fails to explain why its incidence is higher among Orientals. Study of the frequency of diploid sperm among Oriental males and that of defective ova among Oriental females could shed light on this question.

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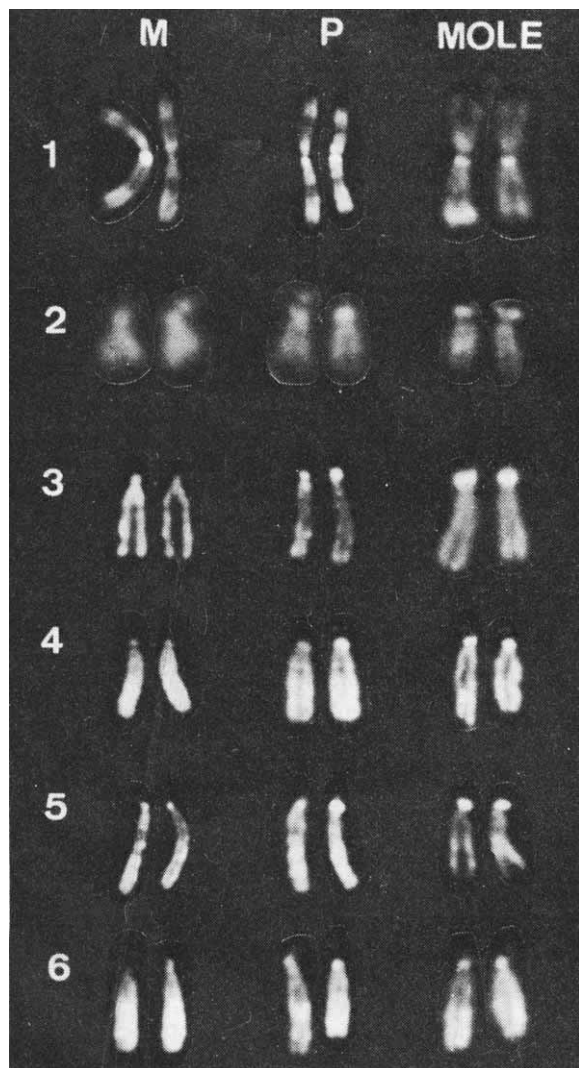


Fig. 2 Chromosomal polymorphisms demonstrating the differences between the maternal (M), paternal (P) and molar (mole) chromosomes. Cases 1, 2, 4, 5 and 6: Q-banding. Case 3: R-banding. Case 1: no. 3. Case 2: no. 22. Case 3: no. 14. Cases 4-6: no. 13. In each case, a paternal chromosome (right) was transmitted in duplicate to the mole, while neither of the maternal homologues was inherited by the mole.

Genetic model for observed distributions of proportions of haemoglobin in sickle-cell trait

IN individuals with sickle-cell trait, mature erythrocytes contain less haemoglobin (Hb) S than HbA. Although variations in the proportions of HbS and HbA have been recognised since quantification of the Hb fractions first became possible, the mechanisms responsible are incompletely understood. Nutritional deficiencies^{1,2} and other non-genetic conditions^{3,4} affecting globin synthesis may alter these proportions in individual heterozygotes but are unlikely to explain the variations found in population surveys. A simple genetic model which can account for the observed distributions of the proportions of HbS in sickle cell trait is presented here.

For most populations, the observed proportions of HbS in heterozygotes may be grouped into three phenotypic classes

Table 1 Phenotypes and genotypes: model with two genetic units (G_1 and G_2)

Phenotype (% HbS) Mean	Range	Model genotype	Frequencies
25	(20–29)	G_1G_1	p_1^2
33	(30–36)	G_1G_2	$2p_1p_2$
40	(37–50)	G_2G_2	p_2^2

with mean values of 25% (range 20–29%), 33% (range 30–36%), and 40% (range 37–50%). The model assumes that these values are determined by two types of genetic units (explained below), G_1 and G_2 , such that the phenotypes with 25, 33 and 40% of HbS correspond to the G_1G_1 , G_1G_2 and G_2G_2 genotypes. If the respective frequencies of G_1 and G_2 are p_1 and p_2 then for a population in Hardy-Weinberg equilibrium, the genotype frequencies are given by the binomial expansion $(p_1 + p_2)^2$ as

shown in Table 1. Given the number of individuals observed in each phenotypic class, the gene frequencies p_1 and p_2 , and the number of individuals expected in each class may be calculated⁵. The goodness of fit of these expected frequencies with observed phenotype frequencies may be evaluated with the χ^2 test⁶.

Ten studies (refs 6–14 and G. Brittenham *et al.*, unpublished) have described the distributions of the proportions of HbS in 13 populations. Table 2 displays the observed and expected numbers of individuals in each of the phenotypic classes, the calculated gene frequencies for G_1 and G_2 , p_1 and p_2 , and χ^2 values derived from the data contained in these reports. Since these studies have used different methods of quantifying the Hb fractions, the range of the phenotypic classes varies slightly by study. The results show an excellent fit between these observations and the predictions of the model; none differs significantly from the expected distribution. Thus, the proportion of HbS in heterozygotes in these populations may be considered to be binomially distributed.

What do G_1 and G_2 represent? One hypothesis would suggest that they are β -thalassaemia-like genes which selectively diminish β^S but not β^A production; a binomial distribution of the proportions of HbS would result. No β -thalassaemia gene affecting the synthesis of a β -chain structural mutant has, however, been definitely demonstrated. Moreover, higher HbA₂ levels might be expected but only modest elevations have been observed in heterozygotes with low proportions of HbS. There seems to be no evidence to support this possibility. A second hypothesis proposes that variations in the number of β -globin gene loci account for variations in the proportion of HbS in heterozygotes¹⁵. Both the available genetic data^{16–18} and direct measurement of the number of β -globin genes¹⁹ indicate, however, the existence of only a single β -chain locus.

The simplest remaining explanation is that G_1 and G_2 represent the number of active α -globin structural genes. Under this hypothesis, G_1 and G_2 would be chromosomes with

Table 2 Observed distributions of the proportions of HbS in sickle-cell trait and Hardy-Weinberg expectations: various populations

Reference	Population	Number		G_1G_1	Genotypes G_1G_2	G_2G_2	'Gene' frequencies p_1	p_2	χ^2
6	US negroes	70	Obs.	HbS < 30%	HbS 30–38%	HbS > 38%	0.279	0.721	0.066
			Exp.	5	29	36			$P > 0.70$
7	US negroes	69	Obs.	HbS < 30%	HbS 30–35%	HbS > 35%	0.152	0.848	1.712
			Exp.	3	15	51			$P > 0.10$
8	Brazilian negroes	60	Obs.	HbS < 30%	HbS 30–38%	HbS > 38%	0.158	0.842	2.100
			Exp.	1.6	17.8	49.6			$P > 0.10$
9	Brazilian negroes	272	Obs.	HbS < 30%	HbS 30–38%	HbS > 38%	0.077	0.923	0.104
			Exp.	3	13	44			$P > 0.70$
10	Brazilian negroes	153	Obs.	HbS < 30%	HbS 30–36%	HbS > 36%	0.101	0.899	1.944
			Exp.	1.6	38.8	231.6			$P > 0.10$
11	Nigerian negroes	244	Obs.	HbS < 28%	HbS 28–36%	HbS > 36%	0.490	0.510	2.795
			Exp.	52	135	57			$P > 0.05$
12	a Nigerian negroes	79	Obs.	HbA > 70%	HbA 65–70%	HbA < 65%	0.709	0.291	0.144
			Exp.	39	34	6			$P > 0.70$
	b Ghanaian negroes	63	Obs.				0.532	0.468	0.169
			Exp.	17	33	13			$P > 0.60$
13	Saudi Arabian caucasians	109	Obs.	HbS < 30%	HbS 30–38%	HbS > 38%	0.578	0.422	0.308
			Exp.	35	56	18			$P > 0.50$
14	Indians (Irula)	79	Obs.	HbS < 30%	HbS 30–35%	HbS > 35%	0.943	0.057	0.288
			Exp.	36.4	53.2	19.4			$P > 0.50$
*	a Indians (Mulla Kurumba)	55	Obs.	HbS < 30%	HbS 30–34%	HbS > 34%	0.400	0.600	1.023
			Exp.	7	30	18			$P > 0.30$
	b Indians (Paniyan)	39	Obs.				0.821	0.179	0.654
			Exp.	8.8	26.4	19.8			$P > 0.40$
	c Indians (Sholiga)	22	Obs.				0.977	0.023	0.012
			Exp.	27	10	2			$P > 0.90$
				26.3	11.5	1.3			
				21	1	0			
				21.0	1.0	0			

*G. Brittenham *et al.*, unpublished

1 and 2 active α -globin loci, respectively. G_1G_1 would correspond to an α^A/α^A genotype, G_1G_2 to $\alpha^A/\alpha^A\alpha^A$, and G_2G_2 to $\alpha^A\alpha^A/\alpha^A\alpha^A$. A net α -chain production deficit would be found in individuals with less than 4 α genes. In at least some cases, α -thalassaemia (α -chain production deficit with unbalanced α/β synthesis) results from deletion of the α -chain gene locus. It has been demonstrated that a low proportion of HbS is found in some heterozygotes with α -chain deficiency due to α -thalassaemia^{20,21}. Calculation of the absolute amounts of the Hb fractions per cell (MC[HbA] and MC[HbS]) demonstrates that the absolute amount of HbA does not differ significantly between those with high or low percentages of HbS. Only the amount of HbS (MC[HbS]) is decreased in those with a low percentage of HbS²². This result is evidence that α chains have a greater affinity for β^A chains than for β^S chains, a hypothesis that was suggested over a decade ago²³ and is supported by recent *in vitro* data²⁴. Thus, the proportion of HbS can reflect the availability of α chains, which can be, in turn, a function of active α -globin gene number.

α -Thalassaemia may not be an invariant finding in those individuals with a low proportion of HbS. A recent study has presented evidence that α/β synthesis may be balanced in individuals with two or three α -globin genes²⁵. Furthermore, balanced globin synthesis has been reported in two heterozygotes with 25 and 28% HbS¹¹. In the absence of other conditions affecting globin synthesis, a low proportion of HbS can indicate a deficit in α -globin chains due to a decreased number of active α -globin structural genes, with or without an associated imbalance in globin synthesis (α -thalassaemia).

If the proposed interpretation of the genetic model is correct, there should be a close correspondence between model predictions of α^A and $\alpha^A\alpha^A$ gene frequencies and estimates by other techniques. The level of Bart's Hb (γ_4) in cord blood specimens has been used to estimate the number of α -chain loci. In the same US black population reported in study 2 (ref. 7; Table 2), analysis of 14,058 cord blood samples has provided frequencies of 0.162 for α^A and 0.838 for $\alpha^A\alpha^A$ genes²⁶. These values are almost identical to the estimates of 0.152 for α^A and 0.848 for $\alpha^A\alpha^A$ derived from analysis of the observed distributions of the percentages of HbS (Table 2). Other evidence, including measurement of the proportion of α -chain variants¹⁸, the number of α -chain variants in certain individuals¹⁸, and direct measurement of the number of α -globin genes in DNA hybridisation experiments¹⁹, indicate that the α -chain locus is duplicated in some populations. Finally, recent family studies have provided further evidence that the proportions of β -chain variants in heterozygotes are a function of the number of α -chain loci²⁷.

The existence of a chromosome with no active α -chain loci has recently been demonstrated in American blacks^{26,28} and is well documented in Asians²⁹. The genetic model may be extended by letting G_0 , at frequency p_0 , represent this chromosomal condition. For a population in Hardy-Weinberg equilibrium and having three types of chromosome, with no, one and two α -chain loci, the genotype frequencies are given by the binomial expansion $(p_0 + p_1 + p_2)^2$ as shown in Table 3. The distributions of the proportions of HbS reported for the heterozygotes of 11 of 12 Guinean and Angolan populations³⁰ fit the predictions of the three chromosome type model (data not shown, available from the author). This result must be interpreted with caution, however, because

the conditions of this study³⁰ precluded detection of hydrops fetalis, HbH disease or α -chain variants.

The genetic model presented here suggests that human populations are polymorphic for the number of active α -globin genes. With differing proportions of HbS in sickle-cell trait, the absolute amount per cell of HbS, but not HbA, varies, supporting the *in vitro* evidence that α chains have a greater affinity for β^A chains than for β^S chains. The proportion of HbS can, therefore, reflect the availability of α chains which, in turn, can be a function of the number of α -globin genes. By using the proportion of HbS as an indicator of α -globin number and postulating that populations are in Hardy-Weinberg equilibrium for chromosomes with two, one and no α -chain loci, the distribution of the proportion of HbS can be accounted for in 24 of the 25 populations reviewed.

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Absolute visual threshold and scotopic spectral sensitivity in the tawny owl *Strix aluco*

It has long been assumed from considerations of the behaviour of owls that the vision of these species is much more sensitive than that of man. Experimental determinations of the absolute visual sensitivity of these birds have provided evidence in support of this view, suggesting that visual sensitivity in owls is of the order of 10 to 100 times ($+1.0 \log_{10}$ to $+2.0 \log_{10}$ units) that of man^{1,2}. Owing to calibration problems, however, these values were probably inaccurate by at least a factor of 10 (refs 1, 2). I report here that in at least one highly nocturnal owl species, visual sensitivity is only slightly higher than that of man, and that the increased sensitivity can be attributed to optical factors rather than to any increase in sensitivity at the retinal level. This suggests that retinal mechanisms in both man and owl have reached the ultimate in sensitivity for the duplex retinas of terrestrial vertebrates.

Determinations of human absolute sensitivity in ideal experimental conditions suggest that once a photon has been absorbed by the photoreceptors, the human retina

Table 3 Phenotypes and genotypes: model with three genetic units (G_0 , G_1 and G_2)

Phenotype (% HbS)	Model genotype	Frequencies
Mean Range		
(Hydrops fetalis)	G_0G_0	p_0^2
17 (15-19)	G_0G_1	$2p_0p_1$
(HbH disease)		
25 (20-29)	G_0G_2 , G_1G_1	$2p_0p_2 + p_1^2$
33 (30-36)	G_1G_2	$2p_1p_2$
40 (37-50)	G_2G_2	p_2^2

comes close to the theoretical limit of sensitivity^{3,4}. Hecht, Shlaer and Pirenne⁵ have shown that single photoreceptors can respond to the absorption of a single quantum of light and that the retina as a whole is capable of detecting small brief stimuli when only about 10 light quanta are absorbed by the receptors. The emphasis in these figures is, however, on absorbed quanta and a sensitivity increase over that of man could be achieved at the retinal level by increasing the probability of quantum catch in the receptors. This could be effected by enlarging the photoreceptor outersegment and/or by increasing the density of pigment contained therein⁵.

Considerable improvement in sensitivity of the eye as a whole, as opposed to that of the retina alone, could be achieved by an increase in the relative proportion of quanta falling upon per unit retinal area for the given stimulus external to the eye. (Approximately 90% of quanta incident on the human cornea are absorbed or scattered before reaching the retinal receptors^{4,6}.) This could be achieved by increasing the light gathering power (photographic speed) of the eye and by increased transmission of light through the optic media.

I determined the absolute sensitivity threshold to achromatic light and the scotopic spectral sensitivity of two tawny owls, *Strix aluco*, using a training procedure designed to produce a high degree of behavioural control by the visual stimuli close to threshold. For comparison, the absolute thresholds of two human subjects were determined in as near identical conditions as possible.

The owls were maintained as described previously⁷, and a two-choice simultaneous discrimination procedure with appetitive reinforcement was used. The training procedure and apparatus were modified versions of those used previously with the same subjects^{7,8}. One important modification was the addition of a gentle air flow directed towards the bird around the response bars while a trial was in progress, and around the food dish during reinforcer presentation. This air flow was used as a cue to increase behavioural control of the animals while responding in total darkness⁹. All surfaces inside the response chamber were painted matt black. The whole apparatus (which was mounted in a light-proof room) was made light tight and during training was covered by a large bag of heavy gauge black polythene sheeting.

Table 1 shows absolute threshold data for achromatic stimuli for the owls and humans after different length dark-

adaptation periods. For the owls training and testing lasted approximately 12 months. Sessions in which no light was presented produced a chance level of discriminative performance suggesting that the animals' behaviour was not under the control of stimuli other than the stimulus lights.

As found in other vertebrates dark adaptation in the owl is close to asymptote after 30 min. Comparison with previously published⁴ human absolute threshold data for extended light sources (mean of 22 subjects = 0.75×10^{-6} cd m⁻² range $0.4\text{--}2.0 \times 10^{-6}$ cd m⁻²) shows that the human thresholds recorded here were within the normal range. Clearly, the behaviourally determined absolute sensitivity of the owl is not as superior to that of man as has commonly been supposed. The normal range within which human absolute thresholds lie covers approximately 1.0 log₁₀ unit, and the data presented here suggest that while the absolute threshold of the owl is probably below that of man the two species show considerable overlap in the distribution of their thresholds. The broadness, compared with that of man, of the owl scotopic spectral sensitivity function (Fig. 1), which has also been found electroretinographically¹¹, is probably attributable to the slightly increased *in situ* rod visual pigment density in the owl. The spectral locus of maximum sensitivity corresponds with the locus of maximum absorption of owl rod visual pigment (λ_{max} 503 nm). (Bowmaker, J.K. and Martin, G.R. in preparation).

It does not seem that the increase in owl sensitivity compared with that of man, is attributable in any large measure to differences in either pigment density or retinal neural mechanisms in the two species. Consideration of the light-gathering power of the tawny owl's tubular shaped eye suggests that as in the cat¹² (where the position is complicated by the presence of a tapetum) the difference in the light-gathering power of the human and owl eye is sufficient to account for the observed difference between owl and human absolute sensitivity. The minimum *f*-number (maximum pupil diameter) of the owl eye is 1.30 (in preparation), while that of man^{13,14} is 2.10 producing a retinal illumination in the owl approximately 2.6 times that of man (compare mean absolute sensitivity difference (two owls compared with two humans) of approximately 2.5-fold). Thus it can be concluded that the human extrafoveal retina is of approximately equal sensitivity to that of other vertebrates considered to be highly nocturnal. The data also suggest that the human retina, along with those of the cat and the owl, has indeed attained the absolute limit of

Table 1 Absolute visual sensitivity threshold data for two humans and two tawny owls following various dark-adaptation periods

	Dark-adaptation period (min)	Threshold log ₁₀	Mean log ₁₀	Mean threshold calibrated (cd m ⁻²)	Threshold relative to human
Owl (01)	30	3.54	3.56	0.84×10^{-6}	$\times 1.45(+.16 \log_{10})$
	60	3.64			
	90	3.49			
Owl (02)	30	3.96	4.04	0.28×10^{-6}	$\times 4.36(+.64 \log_{10})$
	60	4.17			
	90	4.00			
Human <i>a</i>	30	3.50	3.40	1.22×10^{-6}	
Human <i>b</i>	30	3.38			

Thresholds are shown in the arbitrary log₁₀ units from which the calibrated values were determined. Subjects were dark adapted in the apparatus. The only light present throughout training and testing was the stimulus light. Length of stimulus presentation was determined by the latency of the subjects' response and was rarely more than 5 s even during blank probe trials when no stimulus light was presented. Reinforcers were presented during 10-s intertrial intervals signalled by a buzzer. Forty trials per daily session were used with a left-right Gellerman sequence and stimulus intensities presented in a quasi-random order according to a method of constant stimuli. Psychophysical functions (% trials correct per stimulus intensity) were constructed for each subject using between 40 and 100 trials per intensity and these were interpolated at the 75% correct point to give threshold values. Threshold data were accumulated after extensive training. The human subjects (male aged 27 yr; female, 26 yr) viewed the stimuli without fixation through a hole cut in the back of the owls' response chamber (stimulus subtense, $23 \times 15^\circ$) and responded by closing a microswitch. Extensive practice was given. Fifty trials were recorded at each stimulus intensity and thresholds determined as for the owls. The stimulus light (correlated colour temperature 2850 K) was provided by a quartz halogen lamp (GEC 450, under-run from a constant current supply) back projected onto 12×8 cm (angular subtense at bird's perch, $48 \times 34^\circ$) ground glass panels by an optical system similar to that described by Muntz and Northmore¹⁰. Light entered the apparatus through a 5-mm diameter hole (placed at the focal point of one of the lenses) in a light-proof box mounted behind the stimulus panels.

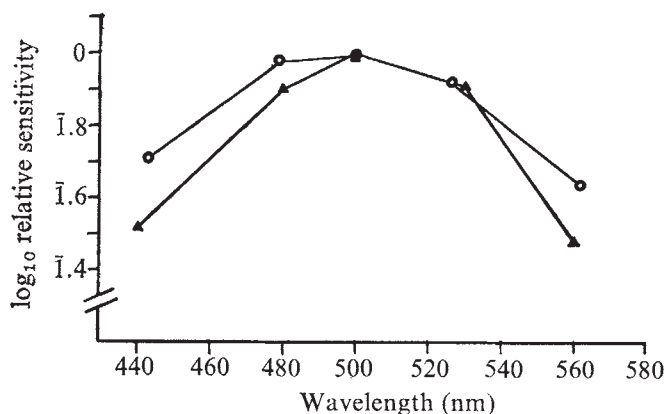


Fig. 1 Behaviourally determined scotopic spectral sensitivity of the tawny owl (○) (mean of two birds). Thresholds were determined using interference filters and the same procedure and apparatus as in the determination of absolute sensitivity to achromatic stimuli. For comparison the human CIE V_{λ}' function is also shown (Δ). Sensitivity is expressed according to an equal quantum intensity base. For calibration of stimuli the following were used: an Hilgar-Shwartz F.T. 17 thermopile placed at the first focus of the optical system to determine the relative energy available through the interference filters (Balzers, B40) and the density of high value neutral density filters *in situ*; an EMI photomultiplier with entrance window placed against the stimulus panels was used to determine the *in situ* density of the low value neutral density filters; a Shimadzu scanning spectrophotometer to determine the spectral transmission of the interference filters. An SEI photometer was used to determine the absolute luminance values of the stimulus panels with filters removed from the beam, and stimulus values at threshold were determined by calculation from the calibrated filter values.

sensitivity, at least for those terrestrial vertebrates with duplex retinas in which some kind of pattern vision is required at low light levels.

Man is typically used as the yardstick for interspecies comparisons of sensory capacities. To gain some appreciation of the adaptive significance of such capacities for the owl, however, a comparison with man is not particularly appropriate. Of more value is the comparison of the owl's sensitivity with that of a diurnal bird, such as the pigeon, *Columba livia*. This species, which is known to roost immediately on being placed in darkness, has an absolute sensitivity approximately $2.0 \log_{10}$ unit (that is, 100-fold) lower than the owl¹⁵. This difference in sensitivity is probably attributable to a difference in retinal mechanisms since retinal illumination in the pigeon is approximately equal to that of man¹⁶ (from observation of pupil diameter). Thus, although the vision of owls does not seem to be as highly sensitive as previously supposed, the sensitivity of these birds can be seen nevertheless to provide them with considerable behavioural advantage over other (diurnal) avian species at low light levels.

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Maternal effects determine effective lethal phase of carnation-light synthetic lethal in *Drosophila melanogaster*

GENETIC conditions which preclude development to the reproductive stage (lethal factors) include dominant and recessive Mendelian units, genomic imbalance and gene interaction. Determination of the ontogenetic phase at which each lethal factor inflicts atypical development and death (effective lethal phase¹) provides an opportunity for study of specific genetic requirements for normal development. I describe here maternal effects which determine the effective lethal phase (ELP) of a synthetic lethal² produced by the interaction of two non-lethal eye-colour mutations, *carnation* (*car*) and *light* (*lt*), in *Drosophila melanogaster*.

Penetrance of the lethal interaction of the *car* (position 62.5 on the X chromosome) and *lt* (position 55.0 on the second chromosome) genes is complete such that all *car-lt* double mutants (*car/car; lt/lt* and *car/x; lt/lt*) die before eclosion. Both mutations cause a general reduction inpteridines and ommochrome precursors in larvae and adults when compared with wild type. Compound accumulations are similar to wild type during pupal stages³.

The influence of the maternal genotype on the ELP was determined by examining the stage of developmental arrest of *car-lt* offspring from matings in which the number of *car*⁺ and/or *lt*⁺ genes varied in the mother. As Fig. 1 shows, a general trend is apparent—as the number of maternal *car*⁺ and *lt*⁺ genes increases, the later developmental arrest occurs in *car-lt* offspring. Yet, a comparison of data presented for crosses IIa and IIb indicates that the stage of developmental arrest is not solely dependent on the number of maternal *car*⁺ and/or *lt*⁺ genes. Females homozygous for *lt* produce synthetic lethal offspring which die at early pupal stages (cross IIb), whereas those from females heterozygous at *car* and *lt* loci die at late pupal stages. A synergic relationship therefore exists between maternally derived *car*⁺ and *lt*⁺ gene products. The difference in ELP in offspring from crosses Ib and IIb suggests that the *car*⁺ gene is semidominant with respect to lethal phase determination. The appearance of the semidominant behaviour of *car*⁺ in crosses IIa and III may be masked by the fact that the late pupal stage is the final stage obtainable by *car-lt* double mutants regardless of the maternal genotype. A test cannot be made of the dominance relations of *lt*⁺ with respect to survival of synthetic lethal offspring. A comparison of crosses Ia and Ib indicates that maternal *car*⁺ and *lt*⁺ gene products have a similar influence on determination of the lethal phase of larvae. The relative infrequency of second-instar lethality is in agreement with other investigations^{4,5}. No differences between sex and lethal phases were observed in any cross. Whether activity of maternal *car*⁺ or *lt*⁺ genes eliminates the cause of larval death of *car-lt* double mutants or merely prolongs the onset of a common developmental crisis is not known. Synthetic lethal larvae from crosses Ia and Ib show no gross abnormalities. Pharate adults from crosses IIa and III, however, show abnormal development of internal genital disk derivatives (males) and incompletely developed abdominal tergites (males and females).

It is generally agreed that each lethal factor defines a particular ELP (monophasic) as embryonic, egg-larval

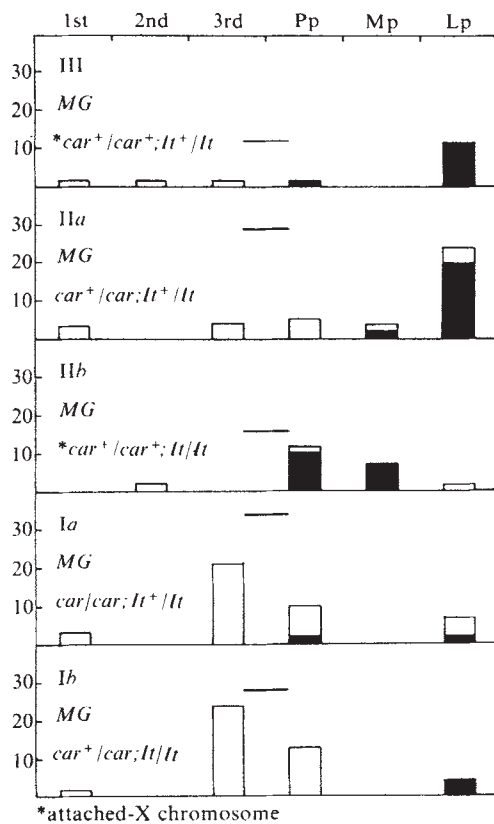


Fig. 1 Number of *car-lt* double mutants (shaded) and unclassifiable (open) offspring dying at various stages from crosses in which the maternal genotype (MG) included various numbers and/or combinations of *car*⁺ and/or *lt*⁺ genes. The paternal genotype was *car*/*y*; *lt*⁺/*lt* (plus appropriate marker genes described below) for each cross. Eggs collected from females (aged 2–6 d) were placed in culture tubes (6 × 50 mm), two per tube, containing 0.1 ml of standard medium. Each tube was examined periodically and scored as to condition of eggs (hatched or unhatched), larvae (instar: 1st, 2nd or 3rd) and pupae (stage). Pupae which failed to eclose within 15 d of egg collection were scored as to the stage of developmental arrest: prepupa (Pp), midpupa (Mp), pupation to onset of bristle pigmentation), late pupa (Lp, bristle pigmentation to pupa ready to emerge). Dead larvae, Pp, or pupae were classified as to genotype when possible by appropriate marker genes (*y*, *lt*, *B*, *f*, *Cy*) as described by Lindsley and Grell¹². Fertility ratios were determined thus allowing computation of the expected number of *car-lt* double mutants for each cross. This number is represented by a horizontal line (—) in each component of the figure.

(boundary), larval-pupal (boundary) or late pupal⁴ in which the degree of genetic change is inversely related to the duration of survival⁶. A direct relationship between genetic complement and time of death was demonstrated by Shannon *et al.*⁷ where a consistent ELP is observed within a given genetic complementation group. A multiphasic lethal pattern is sometimes observed when environmental factors (crowding and so on), genetic background, variations in penetrance and maternal genotype cause some individuals to die at a stage earlier than the characteristic lethal phase^{4,6–8}. Li⁹ reported maternal effects determining survival of all offspring of *Plexate-1*, *plexus* and haplo-IV females. Each case, however, seems to be related directly to the mother's ability to produce functional eggs of all Mendelian classes. Because fertility ratios computed in this study show no consistent relationship to the number of maternal *car*⁺ or *lt*⁺ genes, it can be assumed that the ability of the females used to produce functional eggs *per se* is unrelated to the time of developmental arrest in *car-lt* double mutants. Rather, when sufficient contributions are made by maternal *car*⁺ and *lt*⁺ genes, *car-lt* double mutants transcend the larval lethal phase (crosses IIa, IIb and III)

and enter the radically different developmental phase of metamorphosis.

Maternally derived *lt*⁺ gene products which alter Malpighian tube pigmentation in *lt/lt* offspring from *lt*⁺/*lt* mothers¹⁰ may be involved in lengthening survival time in *car-lt* double mutants. It is improbable, however, that these products directly lengthen survival beyond pupation (as in crosses IIa and III), for the maternal influence on pigmentation ceases before pupation¹¹. The chromatographic-fluorometric procedure used to assess the pigment maternal effect resulting from activity of the *lt*⁺ gene was used to determine whether a similar maternal effect is associated with *car*⁺. Results were inconsistent with such an effect.

The viability of *car*/*car* or *lt/lt* genotypes indicates that their alleles (*car*⁺ or *lt*⁺) are not necessary for normal development unless homozygosity for the mutant allele exists at the other locus. In these cases survival depends on the presence of *car*⁺ or *lt*⁺ gene products by at least the early third-instar stage. In *car-lt* double mutants these products are maternally supplied whereas in survivors (*car*⁺/*car*: *lt/lt* or *car/car*: *lt*⁺/*lt* for example) these gene products are supplied by the organism's own genome as well as that of the mother.

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Age- and region-dependent concanavalin A reactivity of chick wing-bud mesoderm cells

MORPHOGENETIC potential of embryonic chick limb mesoderm cells declines with time beginning at approximately stage 24 of development¹. This decline occurs in a proximo-distal direction². Accompanying the loss of morphogenetic potential there is an age-dependent decline in the ability of limb and axial mesoderm to sort from one another in a randomised mixture of cells^{3,4}. Due to the age-dependent decline in both morphogenetic potential and the sorting ability of the limb mesoderm cells, it has been suggested that the decline in morphogenetic potential may be related to changes occurring at the cell surface⁵. Cell surface carbohydrate moieties have been postulated to play an integral part in cellular interactions⁶. Because of their high degree of carbohydrate-binding specificity⁷, plant lectins, such as concanavalin A (con A), *Ricinus communis* agglutinin (RCA), and wheat germ agglutinin (WGA), have been used to monitor cell surface changes which occur during development^{7–13}. These changes manifest themselves as differences in lectin-induced agglutinability of cells, and changes in number and distribution of lectin binding sites on the cell surface. This study was designed to determine if there were cell surface changes accompanying the decline in morphogenetic potential of the limb mesoderm cells, which could be detected by the use of plant lectin probes. We report here that there is a slight increase in RCA-induced agglutination of chick wing-bud mesoderm cells

with increasing developmental age. There is also a significant age-dependent, proximo-distal decline in the susceptibility of chick wing-bud mesoderm cells to con A-induced agglutination between stages 19 and 26 of development. As such, there is a spatial as well as a temporal correlation between the decline of con A agglutinability and the decline of the morphogenetic potential of the chick limb-bud mesoderm cells.

Zwilling has suggested that the events of limb development can be divided into two phases: a morphogenetic phase and a phase of cytodifferentiation¹. Limb mesoderm cells have a set of properties during the morphogenetic phase which are responsible for the establishment of general limb form¹. The ability of limb mesoderm to express these morphogenetic properties or potential declines in a proximo-distal direction from a peak at stage 19–20, until the morphogenetic potential is lost at about stage 26 (refs 1, 2). During the interim period, the morphogenetic potential of the limb mesoderm cells in a single limb bud is region-dependent². Table 1 shows that the agglutination of chick wing-bud mesoderm cells with WGA ($125 \mu\text{g ml}^{-1}$) is nearly complete and not age-dependent. RCA ($125 \mu\text{g ml}^{-1}$) induces slightly greater agglutination of cells from older wing buds than of those from younger wing buds. In contrast, treatment with con A at the same concentration induces a significantly greater degree of agglutination of mesoderm cells derived from whole stage 19–20 wing buds than it does of mesoderm cells derived from whole stage 25–26 wing buds. The disparity between the morphogenetic potential of proximal and distal mesoderm in a single limb bud is greatest at stage 25 of development² (Table 1). Limb mesoderm cells from the proximal third of a stage 25 wing bud are significantly less susceptible to con A induced agglutination than are those from the distal third. Furthermore, the difference in susceptibility to con A-induced agglutination between mesoderm cells from the proximal and distal thirds of the stage 25 wing-bud, is greater than that between cells derived from whole stage 19–20 and 25–26 wing buds. This is not surprising since whole stage 25 and 26 limb buds contain mesodermal cells of relatively high con A agglutinability in their distal region.

Table 2 Specific binding of ^3H -con A to stage 25 wing-bud mesoderm cells

Region of wing bud furnishing mesoderm cells	Specific binding of ^3H -con A (c.p.m. per mg protein)
Distal third	$20,616 \pm 910$ ($n = 5$)
Proximal third	$20,844 \pm 700$ ($n = 7$)

Single cell suspensions of proximal and distal stage 25 wing mesoderm were obtained as described in the legend for Table 1. Binding experiments were performed at 0°C to minimise endocytosis of bound con A. The binding reaction was done in a 1 ml volume containing 5×10^6 cells, $125 \mu\text{g}$ unlabelled con A and $1 \mu\text{Ci}$ ^3H -con A (specific activity 41 Ci mmol^{-1}) (New England Nuclear). Controls contained the same components plus 0.1 M α -methyl-D-glucose (Sigma) to indicate the non-specific binding of con A. The amount of binding observed in the controls was subtracted from the values of total binding to give the amount of specific binding. The tubes containing the reaction components were kept at 0°C for 1 h with mixing at 15-min intervals, after which they were centrifuged at 2,500 r.p.m. for 5 min. After the supernates were removed, the pellets were washed three times in 2 ml of ice cold Tyrode's solution to remove any unbound ^3H -con A. The pellets were then dried and dissolved in 1 ml of 1 M NaOH. Aliquots were taken for scintillation counting and protein determinations by the Lowry method¹⁷. Values are mean $\pm$ s.e.m.

It is not yet possible to draw conclusions regarding a causal relationship between the cell-surface changes (suggested by the decline in con A-induced agglutinability) and the loss of the morphogenetic potential of the limb mesoderm cells. There is evidence, however, of a positive temporal as well as spatial correlation between these two phenomena.

To determine the basis of the differential con A reactivity between the cells from the proximal third and those from the distal third of the stage 25 chick wing bud, the relative con A binding capabilities of the cells from these two regions were assayed using ^3H -con A. These experiments indicate that there is no significant difference between the amounts of ^3H -con A bound by the cells from these two regions (Table 2). This is in contrast to a decline with increasing developmental age, in the amount of con A bound by normal mouse limb mesoderm cells between days

Table 1 Correlation of lectin-induced agglutinability of whole stage 19–20 and stage 25–26 wing-bud mesoderm cells and mesoderm cells from proximal and distal thirds of stage 25 wing buds with their morphogenetic potential

Region and stage ^{1,8} of wing bud furnishing mesoderm cells	%Specific agglutination with RCA*	%Specific agglutination with WGA*	%Specific agglutination with con A*	Morphogenetic potential†
Whole 19–20	$25.2 \pm 0.7\ddagger$	$98.2 \pm 0.2\S$	$64.4 \pm 1.7\parallel$	96.5
Whole 25–26	$33.1 \pm 2.0\ddagger$	$98.7 \pm 0.3\S$	$51.8 \pm 2.2\parallel$	37.8
Distal third 25	—	—	$68.2 \pm 2.1\parallel$	100.0
Proximal third 25	—	—	$44.5 \pm 3.2\parallel$	0.0

*Mesoderm from whole wing buds of stage 19–20 and stage 25–26 white leghorn chicken embryos and mesoderm from the proximal or distal third of stage 25 wing buds was collected by dissecting the wing buds from the embryos in a dish containing Tyrode's solution. The proximal and distal thirds of the stage 25 wing buds were kept separate. The whole wing buds or the proximal or distal segments were transferred to depression dishes in which they were incubated with 2% trypsin (Nutritional Biochem.) + 1% pancreatin (Nutritional Biochem.) in Tyrode's for 15–20 min at 37°C to loosen the ectoderm. The ectoderms were removed, and after washing with Ca^{2+} -free Mg^{2+} -free Tyrode's (CMF), the mesoderm was cut into fragments and incubated in CMF containing 0.02% EGTA (ref. 9) (Sigma) at 37°C for 45 min. After this treatment, the EGTA solution was replaced with Tyrode's containing $10 \mu\text{g ml}^{-1}$ DNase (ref. 9) (DNase-Tyrode's) (Nutritional Biochem.). After transferring the mesoderm fragments to Falcon plastic test tubes containing 2 ml of DNase-Tyrode's, single-cell suspensions were obtained by mild agitation. The number of cells in each suspension was determined with a haemocytometer and adjusted so that after addition of the appropriate plant lectin (Sigma) and its specific sugar inhibitor (Sigma) in DNase-Tyrode's to control cultures, and the appropriate plant lectin in DNase-Tyrode's to the experimental cultures, each gave a final cell density of approximately 2.5×10^6 cells ml^{-1} in a total volume of 3 ml. The final concentration of plant lectin was $125 \mu\text{g ml}^{-1}$. To control for nonspecific agglutination, α -methyl-D-glucose, N -acetyl-D-glucosamine, or α -lactose (all Sigma) were added to control cultures containing con A, WGA, and RCA respectively. The final concentration in the control cultures of these specific inhibitors was 0.1 M . The cells were incubated in siliconised 25 ml Erlenmeyer flasks for 30 min at 37°C in a gyratory shaker water bath, operating at 70 r.p.m. After incubation, samples were examined in a haemocytometer and single cells remaining counted. The disappearance of single cells from the cell suspensions was used as a measure of cell agglutination.

*Percentages of nonspecific agglutination were subtracted from the values of total agglutination to give the percentages of specific agglutination. Values are mean $\pm$ s.e.m.

†Percentage of grafts, containing mesoderm cells taken from specific stage and region, which formed complex limb structures as determined by the assay of Zwilling¹⁶. Values for whole limbs obtained percentages for component parts².

‡Whole stage 19–20 significantly different from whole stage 25–26. (Student's t test, $P < 0.05$).

§Not significantly different (Student's t test).

||Whole stage 19–20 significantly different from whole stage 25–26 (Student's t test, $P < 0.001$).

¶Distal third of stage 25 significantly different from proximal third of stage 25 (Student's t test, $P < 0.001$).

11 and 12 of development¹⁴, and an increase in the number of binding sites for con A with increasing developmental age in embryonic chick neural retina cells between days 8 and 10 of development¹¹. In the chick limb, mouse limb, and chick retina systems, these changes in the number of con A binding sites with increasing developmental age are all accompanied by a decrease in con A-induced agglutinability. Therefore, it seems that some cell membrane or surface property, other than the number of con A binding sites, is responsible for the observed differences in con A-induced agglutinability. The mechanism responsible for the differing response to con A treatment in our system may involve the lateral mobility of the con A binding sites within the plane of the plasma membrane since the clustering of these sites has been shown to accompany increased agglutination in other systems^{11,15}.

Morphogenesis involves the interaction of large numbers of cells. Since a primary site of such interactions is at the cell surface, an understanding of the cell-surface changes which occur during development as well as how they relate to changes in the morphogenetic potential of cells, will be of significance to our understanding of morphogenesis in general.

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Monogamous T helper cell

HUMORAL antibodies are produced by lymphocytes of bone-marrow or bursal origin (B cells). Some antigens are apparently capable of stimulating B cells and inducing a response directly but many other antigens are dependent on the cooperative help of lymphocytes of thymic origin (T cells). There are at least two forms of such cooperation between B and T cells. One type of cooperation is 'non-specific' where T cells once activated by specific antigen release a nonspecific factor which can then facilitate the responses of B cells to certain other non-related antigens¹. The other well studied cooperative mechanism is known as 'specific cooperation' and classically this has been analysed with the use of 'hapten-carrier' systems². The exact nature of this cooperative event is not known. It may be that co-operation occurs through widely diffusible specific factors released by T cells, or alternatively the cooperating T and B cells may need to come into close or direct contact. We have studied helper T-cell function in a microculture system with the aim of determining whether a single helper T cell could activate one or many B cells. Experiments of this

Table 1 Number of clones observed in each well compared with numbers expected for random distribution of T cells

No. of clones	0	1	2	3	4	5
No. of wells observed to contain the following numbers of TNP-specific B-cell clones*	16	26	12	4	1	0
No. of wells estimated to contain the following numbers of TNP-specific B-cell clones if 1 T cell activates 1 B cell†	19.28	21.56	12.06	4.53	1.28	0.28

*Analysis of the number of wells containing different numbers of B cell clones from the 59 samples analysed in Fig. 2.

†If we hypothesise that one T cell activates one B cell then the clones would be distributed according to the Poisson distribution of T cells getting into wells. A total of 66 clones as observed would be expected to distribute as shown.

type have previously been performed by repopulating mice with limiting numbers of immunocompetent cells and examining the resulting foci of antibody activity. When T cells were the limiting population some have found these foci to be heterogeneous³ and others have found them to be monoclonal^{4,5}. Similar *in vitro* studies have also suggested marked restriction in the B-cell response where T helper cells were limiting⁶. Here we present direct evidence to support the findings demonstrating restriction. We conclude that in the conditions used, one specific helper T cell cooperated with one B cell only.

The culture system used in the experiments was the

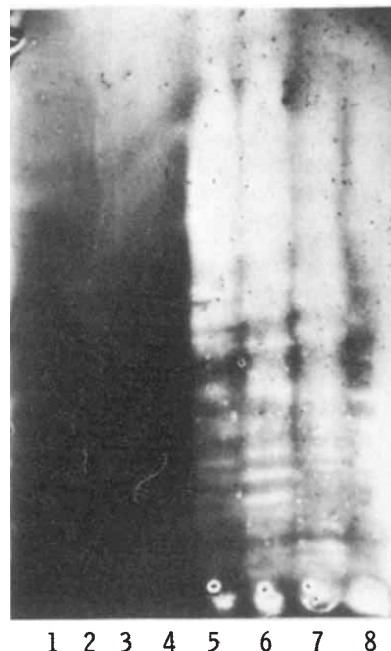


Fig. 1 Anti-TNP spectrotypes obtained from supernatants of *in vitro* microcultures. Antibody was visualised by overlaying the gel with haptenated red blood cells and complement¹⁰. Microcultures were performed as before⁵. B cells were obtained from mice primed three months previously with 500 µg of TNP-ovalbumin in Freund's complete adjuvant and subsequently boosted twice with 200 µg of soluble antigen. The last boost was performed two weeks before the experiment. The spleen cells from these mice were treated with anti θ and complement to remove T cells. T cells were derived from mice primed one month previously with 250 µg of KLH in Freund's complete adjuvant and boosted with 200 µg of soluble KLH 6 d before killing. The spleen cells from these mice were enriched for T cells by passage through nylon wool. 1.5×10^6 B cells were cultured with antigen (TNP-KLH) in multiple wells containing 10 µl of medium. Samples 1–4 were from four cultures having no T cells. Samples 5–8 were from wells having 5×10^4 T cells per well.

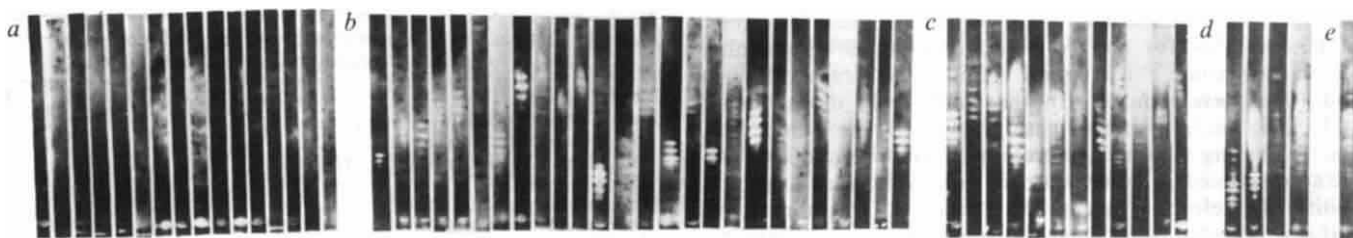


Fig. 2 Anti-TNP spectrotypes from supernatants of *in vitro* microcultures. Antibody visualisation and culture conditions are described in Fig. 1 but in this case 1.5×10^5 B cells and 2.5×10^3 T cells per well were used. 59 individual supernatants were analysed and samples grouped according to the numbers of different spectrotypes (a,b,c,d,e, = 0,1,2,3,4 spectrotypes respectively).

microculture system devised by Lefkovits⁷. The technical procedures adopted for studying the functions of single helper T cells have been described before and it was shown that one single helper T cell was sufficient to activate a B cell response to antigen⁸. Levels of hapten specific B cells were used such that each well contained more than 5 B cells and then T cells were titrated to a level such that many wells would contain 0 or 1 specific helper T cell. After 7 d of culture the supernatants from the cultures were analysed by isoelectric focusing (IEF). By this method different spectrotypes are interpreted as representing the products of different B-cell clones. Figure 1 shows that when no T cells were added all wells were negative, and that when a high number of T cells were added to each well the responses were so heterogeneous that the exact number of clones could not be accurately counted but we can say that there were more than five per well. At a T-cell input of 2.5×10^3 T cells per well, then 16 out of 59 wells which we sampled were negative. With this number of negatives it can be estimated that the majority of the positive wells would contain only one helper T cell. The number of clonal products observed in each of the positive wells of this tray were then counted. In Fig. 2 we show that in 59 wells sampled a total of 66 B-cell clones were generated. If we now hypothesise that these B-cell clones were the result of a 1 T cell : B cell interaction then the clone numbers would be expected to segregate in the wells according to a Poisson distribution of T cells, as these are the cells which are randomly distributed. Table 1 shows the numbers of clones observed in each well compared to that which would be expected by a Poisson distribution of the total 66 clones, and it can be seen that the fit is very close ($\chi^2 = 1.76$). Thus the most accurate estimate we can derive from this data is that one T cell is only able to cooperate with a single hapten specific B cell. The number of wells containing multiple clones can be accounted for by chance distribution of T cells, however, we cannot exclude the possibility that a minority of the T-cell population could cooperate with more than one B cell.

This finding shows that observations which indicated marked restriction in the number of B cells helped by a single T cell were indeed the consequence of a monogamous behaviour of helper T cells. The discrepancies with data showing heterogeneity³ may be the result of the nature of the antigen used since in situations where heterogeneous foci are detected, particulate antigens have been used and with particulate antigens T cells are able to produce a soluble factor (NSF) which can easily replace helper T cells.

The finding that one T cell only reacts with one B cell does not support the idea that this cooperation has occurred through specific factors unless the activity of these factors is short ranged. Either way, the data is not compatible with the concept of widely diffusible specific mediators^{9,10}.

An alternative explanation is that there may be subsets of B and T cells such that each T cell is able to react directly with or release factors which could react with only a certain proportion of the possible B cells. There is some evidence that individual T cells release NSF which is specific

for a subpopulation of the B-cell repertoire¹¹ and it is possible that similar restrictions are placed on specific helper T cells. One situation where specific helper cells have been shown to be selective is in the case of allotype specificity¹². The same selectivity may exist among T cells for immunoglobulin class^{13,14}.

If single T cells could be presented with a large enough range of B cells it should be possible to over-ride any 'matching' restrictions. In our experiments we can only be assured of 5–12 B cells per well and so far attempts to enrich the B cell population for trinitrophenol (TNP) specific B cells have only increased the numbers of hapten specific B cells by two- to fourfold and this did not affect the observed restriction. Enriching for hapten specific B cells would also rule out the possibility that the T cells are not able to 'find' more than a very near B cell.

The third possible explanation for the result is that cooperation between specific T and B cells requires cell-to-cell contact and that once this has taken place the T cell is then, somehow, rendered inactive towards other B cells. Should this in time be shown to be the correct explanation then it must be resolved just how two such rare antigen specific cells (T and B) could locate each other.

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Dissociation of effector functions in populations of activated macrophages

THERE is much evidence to indicate an important role for activated macrophages in resistance to malignant tumours and to infections caused by intracellular pathogens. Different investigators have found that activated macrophages may be distinguished from normal macrophages by either morphological, biochemical, or functional criteria, depending on the

experimental design¹. Functional criteria frequently cited are killing of intracellular organisms^{2,3}, inhibition and/or killing of tumour cells⁴⁻⁶ and inhibition of proliferating lymphocytes^{7,8}. Previous work in our laboratory has demonstrated that macrophages activated by several methods have always exhibited each of these three functional capacities. For example, macrophages activated by either chronic infection with C56 strain *Toxoplasma gondii* or by killed *Corynebacterium parvum* inhibited multiplication of *T. gondii*⁹⁻¹¹, DNA synthesis by tumour target cells^{12,13}, and mitogen- and antigen-specific lymphocyte transformation¹⁴. Whether different populations of activated macrophages which have been defined by one functional criterion always possess the other functional capacities of activated macrophages as well, however, has not been established. We report here that activated macrophages characterised by one functional criterion do not necessarily possess other functional characteristics of activated macrophages and that differences in functional capacity depend on the method used to activate the macrophages.

Three models for activating macrophages were used: *Trichinella spiralis* infection in C57Bl/6 mice (ref. 15 and E.J.W. and J.S.R., in preparation), C37 strain *T. gondii* infection in BALB/c mice¹⁶ and C56 strain *T. gondii* infection in Swiss Webster mice⁹. Peritoneal exudate cells, used as a source of macrophages, were harvested and processed as previously described¹⁷. Macrophage monolayers were challenged with either El-4 tumour cells to test inhibition of tumour cell DNA synthesis¹⁷ or RH strain *T. gondii* to test inhibition of intracellular multiplication¹⁸.

For mice infected with C37 strain *T. gondii*, the dichotomy was observed at 19 weeks after infection.

Macrophages removed from mice infected with C56 strain *T. gondii* and challenged immediately inhibited both DNA synthesis by tumour cells and intracellular multiplication by *T. gondii*. After macrophages had incubated *in vitro* for 24 h, however, the capacity to inhibit DNA synthesis by tumour cells was lost, but the capacity to inhibit intracellular multiplication of *T. gondii* was retained. The density of the macrophage monolayers, which does affect tumour cytostasis, did not change significantly during *in vitro* incubation. Thus, macrophages in the first two experiments inhibited tumour cell DNA synthesis but not intracellular killing, whereas in the third experiment the converse was true.

Our results show that macrophages characterised as activated by one criterion may not satisfy other criteria of activation. That our observations were made in three unrelated experimental systems suggests that this phenomenon is common. The functional differences in different macrophage populations which we have observed may explain the variable results in previous studies and suggest that future publications that discuss activated macrophages should specifically state the criteria used to define the macrophages as activated.

Among the possible explanations for the variability in macrophage functions which we observed is the hypothesis that the functions of activated macrophages in a given population are identical at any one time. The functions of a given population, however, may vary with a number of factors, such as the nature of immunisation and the time after immunisation. Thus,

Table 1 Tumour cell cytostasis and inhibition of intracellular multiplication of *T. gondii* by activated macrophages

Infection used to activate macrophages	Time after infection	Tumour cytostasis (CI)	Infected macrophages with multiplying <i>T. gondii</i> (%)		
			Normal	Experimental	Activated
<i>T. spiralis</i> in C57Bl/6 mice	2 weeks	98	96	98	0
C37 strain <i>T. gondii</i> in BALB/C mice	19 weeks	94	98	98	0
C56 strain <i>T. gondii</i> in Swiss Webster mice					
0 h incubation	16 weeks	99	98	0	—
24 h incubation	16 weeks	-14	99	1	—

Mice infected with *T. spiralis* were used 7–28 d after infection, those with C37 strain *T. gondii* infection at 19 weeks after infection, and those with C56 strain *T. gondii* infection 3–6 months after infection. Macrophages from the C56 strain *T. gondii*-infected mice were tested either immediately or after 24 h of *in vitro* incubation. To test tumour cell cytostasis by macrophages, 8 to 9×10^5 peritoneal exudate cells from appropriate mice were allowed to adhere for 3 h to 6-mm wells of 96-well microtitre plates (Flow Laboratories). The wells were then washed thoroughly with saline to remove nonadherent cells. El-4 tumour cells (2×10^4 in 0.2 ml medium 199 with penicillin 100 IU ml⁻¹, streptomycin 100 µg ml⁻¹, and 10% heat-inactivated foetal calf serum) (M199-FCS) were added to each well. Incubation was carried out for a total of 24 h at 37 °C. Six hours before termination of the culture, the wells were pulsed with from 0.5 to 1.3 µCi (³H)TdR (specific activity 6 Ci mmol⁻¹; Schwarz-Mann). The cells were collected with a MASH II harvester (Microbiological Associates) and then counted in a scintillation spectrophotometer. A cytostatic index (CI) was defined as $((N-A)/N) \times 100$ where N is c.p.m. of tumour cells plus normal macrophages and A is c.p.m. of tumour cells plus activated macrophages. To measure inhibition of intracellular multiplication of *T. gondii* by macrophages, macrophage monolayers were prepared as previously described¹⁷ and then challenged with 2×10^6 RH strain *T. gondii* trophozoites in M199-FCS for 1 h. The monolayers were washed and allowed to incubate in M199-FCS for 18–24 h. The slides were washed, fixed and stained with Giemsa stain. The percentage of infected cells with multiplying *T. gondii* was determined by light microscopy. Normal macrophages were from unimmunised mice; activated macrophages were from mice chronically infected with C56 strain *T. gondii*.

Experiments measuring the ability of macrophages to inhibit tumour cell DNA synthesis and to inhibit intracellular multiplication were run in parallel. In all experiments, macrophages from immunised mice were compared with both macrophages of normal mice (which do not inhibit DNA synthesis of El-4 tumour cells or inhibit intracellular multiplication of *T. gondii*) and macrophages from mice chronically infected with C56 strain *T. gondii* (which inhibit both).

The results are shown in Table 1. Macrophages from both *T. spiralis*-infected mice and C37 strain *T. gondii*-infected mice had the capacity to inhibit tumour cell DNA synthesis. These same macrophages were unable to prevent intracellular multiplication of *T. gondii*. For *T. spiralis*-infected mice, the dichotomy between the two functional capacities was observed in mice infected with from 200–530 larvae (significant mortality occurred above 530 larvae) and from 7 to 28 d after infection.

for example, during an acute infection, when a maximum stimulus to activation occurs, all properties of activated macrophages may exist. These properties might be lost at different rates, and therefore at various times after infection only certain properties of activated macrophages are manifested.

A second hypothesis is that different subpopulations of effector macrophages with different functional capacities exist^{19,20}. Thus, macrophages which inhibit tumour cell DNA synthesis may be distinct from those which inhibit intracellular multiplication. Our observation that in two experimental systems, inhibition of tumour cell DNA synthesis existed alone, whereas in the third inhibition of intracellular multiplication existed alone suggests this second possibility. The complete separation of functions we observed may be more consistent with the existence of different functional populations of activated macrophages.

The separation of the capacity of macrophages to inhibit multiplication by intracellular pathogens and to inhibit DNA synthesis by tumour cells should allow for more exact studies of the functions and biochemistry of activated macrophages.

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Antiviral T cell-mediated immunity in Marek's disease

MAREK'S disease (MD) is a herpesvirus induced lymphoma of chickens characterised by transient virus replication in lymphoid cells and transformation of T cells¹. Several mechanisms probably operate in resistance to Marek's disease, but there is indirect evidence that protective immunity is predominantly T-cell dependent² and comprises both antiviral³ and anti-tumour⁴ immune responses. Thus, a notable feature which distinguishes immunity to Marek's disease from immunity to other herpesvirus infections studied so far is that both effector and target cells are probably T cells and that both virus and tumour-specific antigens may act as targets for immunological attack. Using a plaque inhibition test as a measure of cell-mediated immunity, I show here that T cells obtained from immunised birds are sensitised to virus-specific antigens present at the surface of infected cells of both lymphoid and non-lymphoid origin. The results provide direct evidence for the role of T cells in eliminating infected leukocytes and in reducing the spread of Marek's disease virus (MDV) *in vivo*.

Sensitised lymphocytes were obtained from an inbred line of White Leghorns (Line 7) immunised at three weeks of age by intraperitoneal inoculation of 10^3 plaque-forming units (PFU) of cell-associated attenuated MDV (HPRS-16/att)⁵. Lymphocytes were isolated from peripheral blood by flotation in Ficoll-Hypaque and were washed three times in phosphate-buffered saline before use in plaque inhibition tests. Of the cells obtained, 90%-97% were small lymphocytes and 1.5%-2.0% were monocytes. Two types of tests were carried out. (1) Sensitised cells and MDV-infected cells (target cells) were mixed in suspension and were plated on monolayers of kidney cells derived from Line 7 chickens. Monolayers were washed after 24 h and plaques allowed to develop. (2) Monolayers of kidney cells were pre-infected

with cell-associated MDV for 24 h before addition of sensitised cells. The first test was intended to study direct killing of lymphoid and non-lymphoid target cells. The second was originally designed to study predominantly the effect of sensitised cells on cell to cell spread of virus in monolayers of non-lymphoid cells.

The kinetics of plaque survival with increasing concentration of sensitised cells were consistent with a one-hit process of inactivation. This was noted in both types of tests and for both lymphoid and non-lymphoid targets (Fig. 1). Similar results were obtained in several experiments using sensitised lymphocytes derived from birds 20-70 d after immunisation. These results strongly suggest that sensitised cells act independently in preventing plaque formation and that target antigens are found on both infected lymphoid cells and on infected kidney cells. It has consistently been found that plaque inhibition was 1.5 to 2-fold greater when leukocyte-associated MDV was used as targets as compared with infected kidney cells. The differences observed in both types of tests using sensitised lymphocytes obtained 20-90 d after immunisation were significant ($P < 0.01$ (seven experiments), Student's *t* test). The reason for this difference in susceptibility is not known, but it is possible that kidney-cell associated virus adapted to growth *in vitro* initiates infection more rapidly than leukocytes freshly isolated from infected birds. In this instance, it is known that the spread of MDV from infected leukocyte to kidney cells is delayed in at least a proportion of the cells and it is possible that the probability of plaque inhibition is greater for these cells

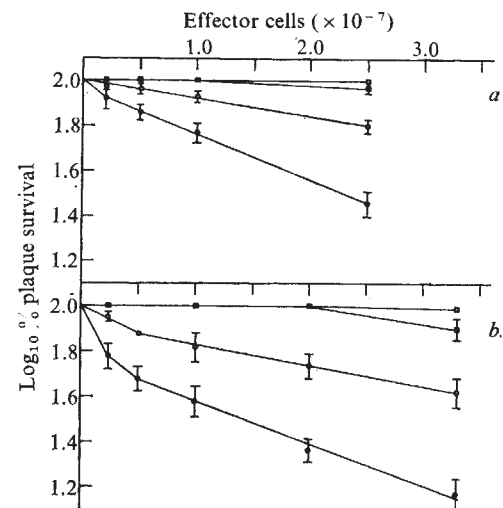


Fig. 1 Kinetics of plaque inhibition. *a*, Aliquots of 10^7 leukocytes obtained from Line 7 birds naturally infected with MDV or of 500 PFU of cell-associated MDV propagated in kidney cells derived from Line 7 birds were mixed in suspension with different numbers of sensitised leukocytes obtained from syngeneic birds. The mixture was plated for infective centres in duplicate on monolayers of syngeneic kidney cells grown in 50-mm Petri dishes. After 24 h at 37 °C, monolayers were washed and fresh medium added. Plaques were counted after 5 d. In this experiment, sensitised lymphocytes were obtained 50 d after immunisation. Lymphocytes used as controls to sensitised lymphocytes were obtained from normal Line 7 birds of identical age. Results shown are means of four estimations using lymphocytes obtained from different birds for each estimation, and are expressed as plaque survival relative to untreated cultures. Bar represents s.e.m. ●, Sensitised lymphocytes + leukocyte-associated MDV targets. ○, Sensitised lymphocytes + kidney cell-associated MDV targets. ■, Normal lymphocytes + leukocyte-associated MDV targets. □, Normal lymphocytes + kidney cell-associated MDV targets. *b*, Monolayers of Line 7 kidney cells in 50 mm Petri dishes were preinfected with leukocyte-associated MDV or with kidney cell-associated MDV. Medium was replaced after 24 h and sensitised or normal lymphocytes added. In this experiment, sensitised lymphocytes were obtained 30 d after immunisation. Cultures were refed after 48 h and plaques were counted 3 d later. Symbols as for Fig. 1*a*.

than for those which allow more rapid transfer of infectious virus. Thus, the survival curve obtained using monolayers pre-infected with leukocyte-associated MDV (Fig. 1b) probably reflects the survival of at least two cell populations differing in susceptibility to killing, each being inactivated with one-hit kinetics.

Although there was evidence of nonspecific inhibition using high concentrations of normal lymphocytes, the inhibitory effect observed at lower concentrations of sensitised cells was virus-specific. Thus, provided that the number of sensitised cells added to 50-mm dishes did not exceed 3×10^7 , plaques formed by MDV only were inhibited. Vaccinia and herpes simplex viruses were not affected. Further evidence for virus specificity was obtained by absorption experiments. For this purpose, sensitised cells were cultured on fixed monolayers of MDV-infected kidney cells or of normal cells. Lymphocytes recovered from normal cell monolayers were still active in plaque inhibition tests whereas those recovered from MDV-infected monolayers were no longer capable of inhibiting plaques (Table 1).

Since sensitised cells were removed by absorption to infected but not to uninfected kidney cell monolayers, it is concluded that plaque inhibition is virus-specific. It follows also that the cells which participate in plaque inhibition are normally non-adherent and that they comprise not more than 1–2% of the population of sensitised cells (Table 1).

The antigens which interact with sensitised cells have not been characterised here, but it is likely that at least some of them might be hitherto unrecognised antigens not detectable using convalescent Marek's disease serum. This follows indirectly from the fact that although Marek's disease serum reacts with antigens at the surface of MDV-infected kidney cells in immunofluorescence tests (17% were positive, Fig. 1a), it does not react with infected leukocytes or with infected leukocytes which have been cultured for 24 h. Yet, infected leukocytes could be prevented from forming plaques after a relatively short time of contact with sensitised cells, and the inhibitory effect was abolished by absorbing sensitised cells to MDV-infected kidney cells. It is possible that some of the antigens which act as targets on MDV-infected leukocytes are analogous to cell surface antigens reported in non-producer EBV positive cell lines of Burkitt's lymphoma⁶. Such cells could be lysed by sensitised T lymphocytes although they did not express serologically detectable cell surface antigens.

To determine the nature of the effector cells which operate in plaque inhibition, sensitised cells were pretreated with antisera specific for chicken T and B cells, and their effect on plaque formation estimated. Significant reduction

in the capacity of sensitised cells to inhibit plaques was noted only when they were pretreated with anti-T serum and complement (Table 2). As the anti-T and anti-B sera have been shown to be cytotoxic predominantly for homologous cells by the trypan blue exclusion test, it is concluded that plaque inhibition is mediated by T cells.

Table 2 Effect of anti-T serum on the capacity of sensitised cells to inhibit plaque formation

Treatment	Experiment 1 Monolayer infected with kidney cell- associated MDV	Experiment 2 Monolayer infected with leukocyte- associated MDV
Sensitised lymphocytes		
+ anti-T + complement	80 ± 3.2*	150 ± 20.1*
+ complement	61 ± 2.3	69 ± 3.0
+ normal rabbit serum		
+ complement	65 ± 5.1	75 ± 4.0
+ anti-B + complement	54 ± 3.7	91 ± 10.4
+ anti-chick Ig		
+ complement	68 ± 4.3	75 ± 8.3
+ anti-T + anti-chick Ig		
+ complement	85 ± 2.6*	141.5 ± 14.9*
Sensitised lymphocytes	60 ± 5	70 ± 8.2
Untreated	80 ± 4.6	160 ± 10.2

Sensitised lymphocytes obtained 50 d after immunisation were treated at a concentration of 5×10^7 per ml with antiserum and complement¹⁰. Antisera against chicken thymus cells (anti-T) and against chicken bursal cells (anti-B) were obtained from rabbits immunised with T and B cells¹¹. Anti-chick Ig was obtained commercially (Nordic). All antisera were used at a final dilution of 1/10. Guinea pig serum absorbed to agarose was used at a final dilution of 1/10 as a source of complement. Treated and untreated sensitised cells were washed twice in RPMI medium + 10% foetal calf serum before use in plaque inhibition tests. These were carried out as described for Fig. 1b with the exception that monolayers were grown in 30-mm dishes and 3×10^6 sensitised cells were used for each dish. Values (means ± s.e.) of plaque counts using lymphocytes from four different birds. Analysis of variance using orthogonal contrast to find the difference between treatment means showed that only treatment with anti-T serum abrogated the capacity of sensitised cells to inhibit plaques.

* $P < 0.001$.

The mechanism of plaque inhibition reported here is different from that reported by others for herpes simplex⁷, infectious bovine rhinotracheitis⁸ and for varicella-zoster⁹ viruses. For these cases, plaque inhibition was attributed to interferon released either as a result of interaction between sensitised peritoneal macrophages and antigen or between sensitised lymphocytes, monocytes and antigen. I was unable to detect interferon-like substances in the medium obtained from cultures in which inhibition of MDV plaques had occurred. Plaque formation by herpes simplex

Table 1 Specificity of plaque inhibition: effect of absorbing sensitised cells to MDV-infected monolayers

Effector cells	Experiment 1		Experiment 2	
	Absorbed to normal cells	Absorbed to MDV-infected cells	Absorbed to normal cells	Absorbed to MDV-infected cells
Control	140 ± 10	124 ± 4	281 ± 9	269 ± 19
MDV-sensitised	72 ± 3	128 ± 10	189 ± 4.5	237 ± 17

Monolayers of normal kidney cells derived from Line 7 birds and of cells heavily infected with kidney cell-associated MDV were fixed with 0.1% glutaraldehyde in phosphate-buffered saline (PBS) for 30 min at room temperature and were thoroughly washed with PBS. Approximately 5×10^7 viable lymphocytes obtained from control birds or from immunised birds 40 d after immunisation were added to the monolayers in 50-mm Petri dishes and were incubated for 3 d at 37 °C in RPMI medium (Flow Laboratories) + 10% foetal calf serum. Non-adherent lymphocytes obtained from the medium and by washing the monolayers once were pooled, washed, and used for plaque inhibition. The proportions of the original number of viable lymphocytes plated recovered from monolayers of normal cells were: 7% (control) and 7.2% (MDV-sensitised). Corresponding values for recovery from MDV-infected monolayers were: 6% (control) and 4.8% (MDV-sensitised). Differences in recovery were not significant except in the case of MDV-sensitised lymphocytes cultured on MDV-infected monolayers (the mean reduction in recovery of 1.2% compared with absorption to normal cells was significant, $P < 0.05$ for both experiments). Plaque inhibition tests were carried out as described for Fig. 1 using 5×10^6 sensitised or control lymphocytes as effector cells. Targets in experiment 1 were monolayers preinfected with leukocyte-associated MDV. In experiment 2, leukocyte-associated MDV targets were mixed with effector cells and were plated for infective centres as described for Fig. 1a. Values are means ± s.e. of plaque counts of four estimations using lymphocytes from four birds for each treatment.

* $0.01 < P < 0.05$.

NS, not significant (Student's *t* test).

was not inhibited when herpes simplex-infected cells, MDV-infected cells and MDV-sensitised lymphocytes were plated simultaneously for infective centres. Similar results were obtained using vaccinia-infected cells (unpublished). Moreover, the cells which participate in plaque inhibition are non-adherent and therefore not macrophages.

Several conclusions can be drawn from these experiments. First, sensitised T cells act independently to inhibit plaque formation. Second, inhibition is probably due to direct lethal interaction between sensitised cells and MDV-infected target cells. Third, hitherto unrecognised virus-specific antigens present at the surface of infected leukocytes may act as targets for sensitised lymphocytes. The plaque inhibition test reported here should prove a better *in vitro* measure of protective immunity than conventional tests for cell-mediated immunity since there is evidence that leukocyte-associated viraemia is invariably reduced in immunised birds compared with controls on infection with virulent MDV (refs 3, 12). A mechanism similar to that reported here for plaque reduction may thus operate *in vivo* to eliminate infected leukocytes and the spread of MDV throughout the tissues of infected birds.

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Heterospecific cytotoxic cell activity induced during the first three days of acute lymphocytic choriomeningitis virus infection in mice

ACUTE infection of adult mice with lymphocytic choriomeningitis virus (LCMV) induces several types of immune responses. H-2-restricted virus-specific cytotoxic T cells are demonstrable from day 5 to 6 and peak on day 7 to 10 post-infection^{1–5}. Antiviral antibody in immune complexes is first detected by 4 d post infection^{6,7}, and non-specifically activated macrophages can be demonstrated after 4 to 5 d (refs 3 and 8). We report here that the acute LCMV infection elicits the production or activation of another type of cytotoxic cell as early as 1 d post infection. These early cytotoxic cells lyse infected or uninfected syngeneic, allogeneic, and xenogeneic cell lines with some specificity. These effector cells do not belong to the cell types thus far implicated in the immune response to LCMV infection and have many of the properties of natural killer cells.

C3H/St (H-2^k) mice were infected intraperitoneally with 2×10^4 plaque forming units (PFU) of the Armstrong strain of LCMV at various days. Their spleens were tested in one test for spleen cell killing of uninfected or LCMV-infected L-929 cells (H-2^b). Figure 1 shows that as early as one day post infection and peaking on the third day post-infection, spleen cells killed uninfected and infected L cell

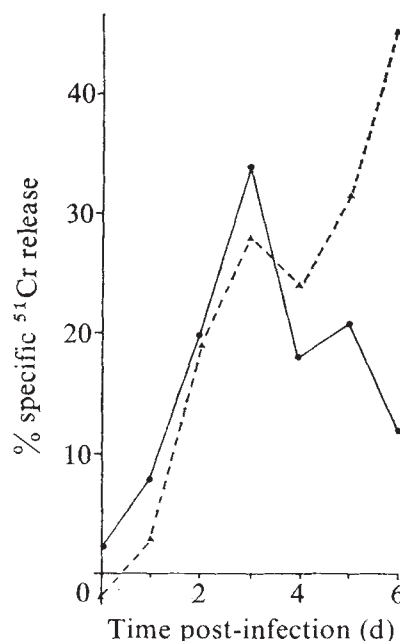


Fig. 1 C3H/St mice were infected intraperitoneally with 2×10^4 PFU of Armstrong strain LCMV. At daily intervals post infection the spleens were collected and cells liberated by gentle squeezing with forceps. Erythrocytes were lysed by exposure to 0.83% NH_4Cl . The erythrocyte-depleted spleen cells were then exposed to 10^4 ^{51}Cr (New England Nuclear) labelled L cells, either uninfected or persistently infected with LCMV²³. In a total volume of 200 μl in microtitre wells (Falcon no. 3040), spleen cells were added at various effector-to-target ratios (100:1, 50:1, 25:1). Data expressed are at effector-target ratios of 100:1. Samples were incubated for 16 h before collecting cells. Background release of ^{51}Cr was that obtained with cells incubated under Eagle's minimal essential medium (MEM). Maximum lysis was determined by exposing labelled cells to 1% NP40. Per cent specific ^{51}Cr release was calculated as

$$\frac{(\text{c.p.m. test sample} - \text{c.p.m. MEM control})}{(\text{c.p.m. NP40 control} - \text{c.p.m. MEM control})} \times 100$$

All assays were run in quintuplicate, and the standard error of this assay method was <5% of the mean. The zero time samples represent spleen cell killing from uninfected mice. The values given here and on subsequent tables represent the average of the mean. ●, L cell target; ▲, L(LCMV) cell targets.

targets with equal efficiency. By the fifth and sixth day post infection, the virus-infected cells were lysed with much greater efficiency than uninfected cells, probably by cytotoxic T cells. During the first 4 d post infection, comparable killing efficiency on a cell-to-cell basis was obtained when peritoneal cells were used as the effector cells. Most subsequent experiments used cytotoxic cells isolated from the spleen or peritoneal fluid of mice at 3 d post infection. Exposure of the LCMV preparation to ultraviolet irradiation⁹ before infection of mice abolished its ability to induce the early cytotoxic cells, and the culture fluid of L cells (used to propagate LCMV) did not elicit the killer cell response. Although most ^{51}Cr release assays were run for 16 h, slightly more than 50% of the killing activity occurred during the first 4 h (Table 1a). No early cytotoxic cell killing was observed if spleen cells were first incubated overnight at 37 °C before exposure to labelled targets (Table 1b). In contrast, the later virus-specific day 8–10 T-cell killing was not reduced by overnight incubation, indicating that the early cytotoxic cells were less stable to *in vitro* incubation than T cells.

The early cytotoxic cells killed a wide variety of cultured syngeneic, allogeneic, and xenogeneic cells. Usually 15–45% of the target cells were killed within 16 h at effector-to-target cell ratios of 100:1. Spleen cells from

C3H/St ($H-2^k$) mice killed L-929 ($H-2^k$), EL-4 ($H-2^b$), P815 ($H-2^d$), J774 ($H-2^b$), and SCRF 179 lymphoblast ($H-2^a$) mouse cell lines, as well as F-9 cells, which have no detectable H-2 antigens¹⁰. They also killed continuous primate cell lines (HeLa, Raji, Wil-2, and Vero), as well as early passage mouse (C3H/St and NIH Swiss), rat (BN and Lewis), hamster (Syrian) and human fibroblasts. In contrast, they did not kill freshly harvested unstimulated peritoneal macrophages or splenic lymphocytes from C3H/St mice. The ability of the early cytotoxic cells to kill such a heterogeneous variety of cultured cells suggested nonspecificity,

Table 1 Stability of early cytotoxic cells in culture % specific ⁵¹Cr release

Experiment a Duration of assay on L cells (h)		1	4	8	12	24
Day 3 effector cells		4.5	18	22	21	23
Control effector cells		-1.0	-1.5	3.0	-0.5	2.5

Experiment b Cytotoxic activity following incubation in culture		Day of spleen cell harvest			
Time of assay after collecting spleen cells (h)	Target	2	4	8	10
0-16	L	16	21	8.8	-0.5
22-38	L	-2.4	-3.4	8.5	1.0
0-16	L(LCMV)	21	29	50	29
22-38	L(LCMV)	-1.3	0.0	64	30

Cells from C3H/St mice taken at the designated times after infection were tested at effector-target ratios of 100 for cytotoxic activity either immediately after harvest (experiment a, b 0-16), or after overnight incubation (experiment b 22-38).

but cold target blocking experiments indicated some degree of specificity (Table 2). Three mouse cell lines with different H-2 specificities and one human cell line (Raji) were exposed to the early cytotoxic cells, and all were lysed at significant levels above controls. Unlabelled cells of each type were then added to the assay wells in sixfold excess of the labelled targets. In all combinations, unlabelled target cells competed best with labelled homologous targets. Non-homologous cells partially blocked the cytotoxicity to various degrees, suggesting some shared target cell specificity.

Early cytotoxic cells were detected after acute LCMV infection in both male and female mice of the following strains: AKR, BALB/c, B10.Br, B10.D₂, CBA/N×DBA/2 F₁, C3H/HeJ, C3H/St, C3H/St×DBA/2 F₁, NZB×NZW F₁ and athymic nude (BALB/c) mice. The latter result with nude mice suggested that the early cytotoxic cell was not a T cell, and this was confirmed by our findings from four experiments that depletion of spleen T cells with antibody to θ antigen plus complement failed to remove the cytolytic activity (Table 3a). Whereas the cytolytic activity of

Table 2 Specificity of early cytotoxic cell % specific ⁵¹Cr release

Effector cell	Blocking cell	EL4*	L929	P815	Raji
Control	None	17	0	1.5	1.5
LCMV	None	44	19	17	25
LCMV	EL4	10	13	11	7.5
LCMV	L929	39	2.6	7.6	16
LCMV	P815	30	15	3.0	16
LCMV	Raji	30	8.1	7.6	1.5

Early cytotoxic spleen cells from C3H/St mice were exposed to ⁵¹Cr labelled cell targets at an effector to target ratio of 100. Unlabelled target cells in sixfold excess of the labelled targets were incubated with the labelled targets in some of the reactions (blocking cell). Spleen cells from uninfected control mice were also tested for their cytolytic activity.

*These EL-4 cells express oncornavirus antigens on their cytoplasmic membranes.

day 8 (T) cells on LCMV-infected syngeneic targets was reduced by 78%, the activity of the day 3 early cytotoxic cells on uninfected targets was enhanced.

Further analyses indicated that the early cytotoxic cell was neither a macrophage nor a polymorphonuclear leukocyte (PML). Most of the cytolytic activity of peritoneal cells isolated from either C3H/St or nude (BALB/c) mice was associated with the non-adherent population (Table 3b) after adsorption to plastic. In three other similar adsorption experiments with spleen cells, the killing activity on a cell-to-cell basis was enriched by 5-25% following removal of adherent cells. When macrophages and PML were removed by passing spleen cells through a glass wool column, the effluent cells, which contained <1% macrophages and <2% PML, retained all of their cytolytic activity.

We found that during the first 3 d after LCMV infection in adult mice, cytotoxic cells appear that kill many types of uninfected or infected cultured cells with some specificity but do not kill primary macrophages or lymphocytes. Pfizenmaier *et al.*^{4,5} have reported that 5 d after infection of mice with LCMV, cytotoxic T cells are generated which kill both infected and uninfected syngeneic targets, and Blanden and Gardner¹¹ have described the appearance of early killer T cells that lysed uninfected syngeneic targets 2-4 d after infection of mice with ectromelia virus. Rodda and White¹² reported a possibly similar early killer cell during Semliki Forest virus infection of mice but concluded that it was a macrophage. The early cytotoxic cell that we describe here is clearly neither a T cell nor a macrophage nor a PML. Cytotoxic cells which are labile at 37 °C and kill tumour cells but not primary cell lines have been reported and designated natural killer (NK) cells. These cells lack surface antigen markers (IgG, Fc

Table 3 Characterisation of early cytotoxic cells % specific ⁵¹Cr release

Experiment a	Effector cell	Depletion of spleen T cells* Target cell	Treatment of effector cell	
			None	Anti- θ +C
Day 3	L		10	13
Day 8	L(LCMV)		63	14

Experiment b Adherence properties of peritoneal killer cells†		C3H/St mouse	
Effector cell			Nude (BALB/c) mouse
Day 3 non-adherent		17.4	22.2
Day 3 adherent		5.5	5.8
Day 3 unseparated		18.5	26.6
Control unseparated		0.4	0.5

*Spleen cells from days 3 or 8 post infection were treated with antibody to θ antigen (a gift from R. Blanden) and guinea pig complement (C), which had previously been absorbed against C3H/St mouse spleen cells. After incubation, the spleen cells were washed and tested on labelled L or L(LCMV) targets at an effector-target ratio of 50.

†Peritoneal cells were adhered to plastic microtitre wells for 2 h at a concentration of 5×10^5 cells per well (effector-target ratio = 50). The non-adherent cells were gently pipetted off the monolayers and transferred to other wells without adjusting the cell number. A monolayer of adherent cells (>80% macrophages) was left. Target L cells were added to the wells for a ⁵¹Cr release assay.

receptors, C3 receptors, θ antigen) and lyse Moloney leukaemia/sarcoma virus infected or transformed cell targets¹³⁻¹⁷. The LCMV infection may activate normally undetectable NK cells that have some additional target specificities. A similar induction of NK cell activity in mice can be elicited by Bacille Calmette-Guerin (BCG) (ref. 18). An alternative hypothesis is that the LCMV infection may induce a polyclonal natural antibody response which would mediate antibody-dependent cell cytotoxicity (ADCC) by K cells, cytolytic lymphocytes with Fc receptors^{19,20}.

Epstein-Barr virus has been reported to induce polyclonal B-cell activation of cultured human lymphocytes²¹. We have been unable to establish a functional role for immunoglobulins or Fc receptors in our system, and little activity is associated with populations of adherent cells (Table 3), known to be effectors of ADCC in mice²². This phenomenon cannot be explained by the transfer of virus with the spleen cells to the labelled targets, rendering them sensitive to anti-LCMV ADCC because (1) there is some target cell specificity, (2) most killing occurs at a time (4 h) before the viral antigens could be expressed on the surface of infected target cells, (3) Raji cells are sensitive targets yet refractory to LCMV infection (R.M.W., unpublished), and (4) very little virus infectivity is transferred in macrophage depleted spleen cell populations. Thus, although we cannot yet completely exclude an ADCC mechanism, our data describing a heat labile, non adherent, non-T lymphocyte active against a variety of cell targets in the absence of added antibody suggest that the early cytotoxic cell generated during the first few days of the LCMV infection is similar to an NK cell.

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Note added in proof: Inductions of NK-like cells in mice by acute virus infections have recently been observed by several other laboratories²⁴⁻²⁶.

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Protein covalently linked to foot-and-mouth disease virus RNA

THE RNA in picornaviruses can function directly as mRNA (ref. 1). In contrast to the great majority of cellular and virus mRNA molecules, however, a 'cap' structure has not been found at the 5'-end of the RNAs of polio^{2,3} and

encephalomyocarditis (EMC) (ref. 4) virus particles or on poliovirus specific mRNA in infected cells^{2,3}. The 5'-terminus of poliovirus mRNA extracted from polyribosomes was found to be pUp^{2,3} but no nucleotide could be identified at the 5'-end of the RNA in virus particles. This is because a small protein is covalently linked to the 5'-terminal nucleotide of the RNA (refs 5, 6). The RNAs of EMC virus and foot-and-mouth disease virus (FMDV) contain a tract of about 100-200 cytidylic acid residues located near the 5'-end^{7,8} but such a sequence was not found in poliovirus RNA (ref. 9). It was of interest, therefore, to determine whether a protein is also covalently linked to FMDV RNA and whether it is attached to the poly C tract. We show here that FMDV RNA is not capped and demonstrate the presence of a protein of molecular weight (MW) about 4,000 covalently linked to the RNA. The protein is not attached directly to the poly C tract but is probably attached to the 5'-terminus and can be removed without loss of infectivity.

When FMDV particles labelled with ³H-uridine and ³⁵S-methionine were extracted with phenol-chloroform in the presence of sodium dodecyl sulphate (SDS), about 0.03% of the ³⁵S counts remained in the aqueous phase together with all the ³H-labelled RNA and could not be removed by repeated extractions with phenol-chloroform. After sedimentation through a sucrose density gradient containing 0.1% SDS, the ³⁵S counts coincided with the ³H-labelled peak of RNA (Fig. 1a). The ³⁵S counts were still associated with the RNA after heating at 70 °C for 1 min in 100% formamide (Fig. 1b), or at 100 °C for 1 min in 5% SDS, 5% mercaptoethanol and 2.5 M urea (Fig. 1c), conditions which would be expected to disrupt non-covalent bonds between protein and RNA. The altered sedimentation profile of the RNA after heating at 100 °C (Fig. 1c) is probably due to non-random degradation.

In contrast, treatment with Pronase in the presence of 0.1% SDS completely removed the ³⁵S-labelled material from the RNA without altering its sedimentation profile (Fig. 1d). These results strongly suggest that the ³⁵S-labelled material is covalently attached to the RNA and is a protein. No ³⁵S-labelled material was associated with the RNA of virus grown in pre-labelled cells, suggesting that the protein is not host specific. Further evidence that the protein and RNA are linked covalently was obtained by digestion with a mixture of T₁ and pancreatic ribonucleases. After this treatment the ³⁵S material remained at the top of a sucrose density gradient demonstrating that it was not fortuitously sedimenting at a rate similar to that of the RNA. The protein is not required for infectivity since an ³⁵S-methionine labelled FMDV RNA preparation with a titre of 1.7 × 10⁶ plaque-forming units per ml and containing 266 c.p.m. per ml had the same titre and sedimentation rate but contained less than 7 c.p.m. per ml after Pronase treatment. This result is in accord with the observation that poliovirus particle and mRNAs have similar specific infectivities despite the lack of a 5'-linked protein on the mRNA (ref. 15).

To characterise the protein the RNA isolated from ³⁵S-methionine labelled FMDV was purified by sucrose gradient centrifugation and then analysed by electrophoresis on 20% polyacrylamide gels¹¹ with or without digestion with a mixture of T₁, T₂ and pancreatic ribonucleases. All the ³⁵S counts in the untreated material remained at the top of the gel but after digestion with the ribonucleases the ³⁵S-labelled material migrated as a band with an apparent MW of about 4,000. Co-electrophoresis of the FMDV RNA-linked protein labelled with ³⁵S-methionine and the corresponding protein from poliovirus labelled with ³H-lysine on high cross-linked polyacrylamide gels¹² showed that they had similar molecular weights.

The 5'-end of the virus RNA was shown to be blocked by analysis of the nucleotides produced by digestion of ³²P-labelled RNA with a mixture of T₁, T₂ and pancreatic

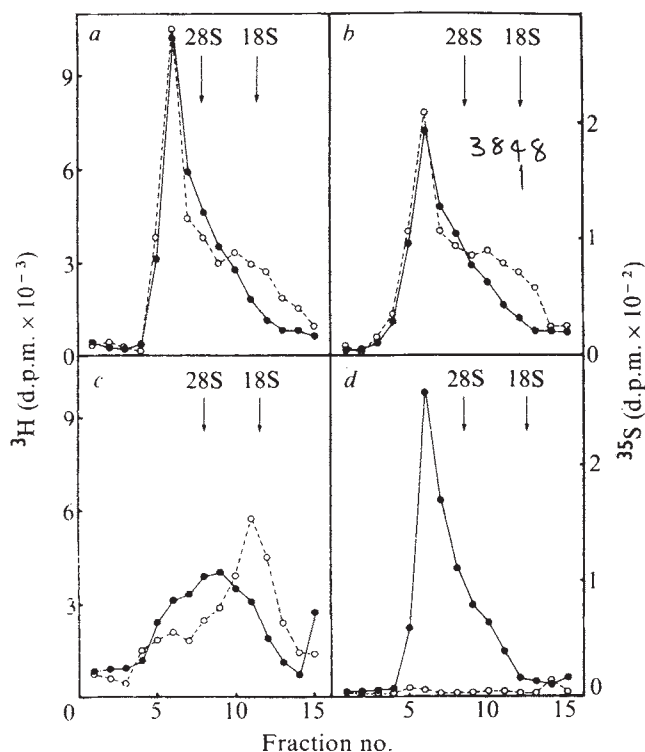


Fig. 1 Effect of denaturing conditions and Pronase on the sedimentation of FMDV RNA. The virus, type A61, was grown in the presence of ^3H -uridine ($0.2 \mu\text{Ci ml}^{-1}$) and ^{35}S -methionine ($25 \mu\text{Ci ml}^{-1}$, Radiochemical Centre, Amersham) and purified by sucrose gradient centrifugation¹⁰. The RNA was extracted with phenol-chloroform-SDS and precipitated at -20°C with ethanol. Aliquots of the precipitated RNA were dried under N_2 and treated as follows before centrifuging at $50,000g$ for 16 h at 20°C through 5–25% sucrose gradients in 0.1 M acetate–0.1% SDS, pH 5.0. The RNA was dissolved in a, 0.5 ml TNE–SDS (0.1 M Tris, 0.1 M NaCl, 0.001 M EDTA, 0.1% SDS, pH 7.5) and heated at 37°C for 1 h; b, 0.1 ml 100% formamide and heated at 70°C for 1 min before diluting to 0.5 ml with TNE–SDS; c, 0.1 ml disrupting solution (2.5 M urea, 5% mercaptoethanol, 5% SDS) and heated at 100°C for 1 min before diluting to 0.5 ml with TNE–SDS; d, 0.5 ml TNE–SDS containing 50 μg Pronase and heated at 37°C for 1 h. BHK ribosomal RNA was then added to each sample to serve as marker before centrifugation. Each fraction was precipitated with 10% TCA in the presence of bovine serum albumin (BSA) and filtered on to a glass fibre disk before counting in a liquid scintillation counter. ●, ^3H ; ○, ^{35}S .

ribonucleases. Chromatography of the digest on DEAE–Sephadex in the presence of 7 M urea showed that there were no nucleotides eluting with a net charge expected for a nucleotide tetraphosphate (–6) or a capping group (–5 to –6) or a structure of the type pXp (–4) or ppXp (–5). This result is consistent with the presence of a free OH or a blocked structure at the 5′-end of the RNA in virus particles.

Further evidence that the 5′-end of the virus RNA was blocked was provided by electrophoresis on Whatman 3MM paper at pH 3.5 of the nucleotides produced by complete ribonuclease digestion. Four mononucleotide peaks were obtained together with small amounts of inorganic phosphate and cyclic mononucleotides, but in addition approximately 0.016% of the ^{32}P migrated towards the cathode. In contrast, less than 0.006% of the ^{32}P moved towards the cathode when the RNA was treated with Pronase before ribonuclease digestion. This suggests that a protein is attached to the 5′-end of FMDV-RNA in the same way that a protein is linked to poliovirus RNA⁸.

As our previous work⁸ had shown that the polyC tract in FMDV RNA is located near the 5′-end it seemed possible that the protein was attached to the polyC tract. To test this possibility the RNA prepared from FMDV labelled

with ^{35}S -methionine and ^3H -cytidine was digested with ribonuclease T_1 and the oligonucleotide products separated by electrophoresis on a 12.5% polyacrylamide gel in 6 M urea at pH 8.5. The ^{35}S -methionine peak did not coincide with the ^3H -cytidine labelled polyC tract (Fig 2a) indicating that the protein was not attached to it directly. After T_1 and pancreatic ribonuclease digestion of ^{35}S -methionine-labelled RNA and subsequent electrophoresis on a similar gel all the radioactivity was found near the top of the gel (Fig. 2b), indicating that the protein does not move to the position shown in Fig. 2a because of its intrinsic size and charge at pH 8.5.

An important feature of the replication of FMDV is that less than 20% of the single stranded (ss) RNA synthesised in infected cells is encapsulated into virus particles¹³. Furthermore, the intracellular FMDV specific ssRNA sediments as a broad band in sucrose gradients with a peak at about 37S, whereas RNA extracted from virus particles sediments at 35S. The amount of protein associated with intracellular FMDV induced ssRNA was found to alter during the growth cycle. Only low levels of ^{35}S labelling were found on RNA up to the time when about half of the total RNA had been synthesised. Thereafter a greater proportion of ^{35}S labelling was observed and this continued to increase after RNA synthesis had virtually ceased. The

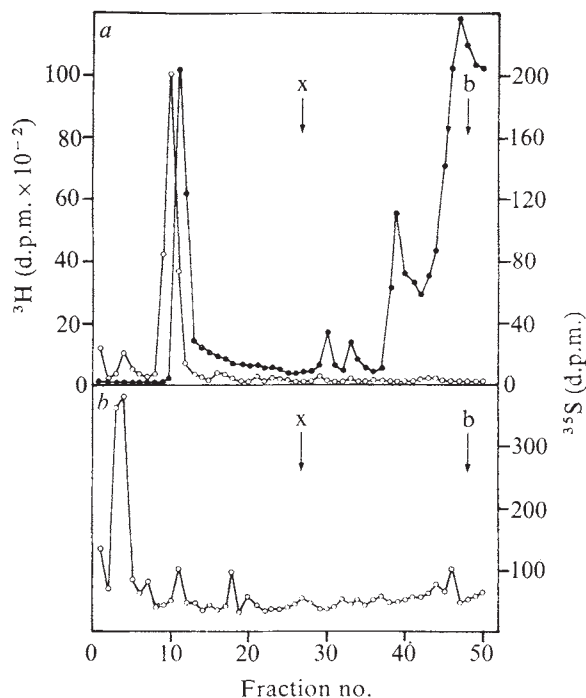


Fig. 2 Polyacrylamide gel electrophoresis of FMDV RNA after digestion with ribonuclease T_1 or a mixture of T_1 and pancreatic ribonucleases: a, Separate preparations of FMDV RNA labelled with either ^3H -cytidine or ^{35}S -methionine were prepared as described under Fig. 1 and purified further by centrifugation on 5–25% sucrose gradients in 0.1 M acetate–0.1% SDS, pH 5.0 at 20°C for 18 h at $50,000g$. The fractions containing the peak of RNA at about 35S were precipitated with ethanol in the presence of 100 μg *Escherichia coli* tRNA as carrier and digested with 30 units of ribonuclease T_1 in 20 μl 0.01 M Tris-HCl 0.001 M EDTA containing 10 μg BSA for 1 h at 37°C . b, 25 μg pancreatic ribonuclease added to the digestion mixture. The digested samples were mixed with an equal volume of dye marker (0.2% xylene cyanol (x), 0.2% bromophenol blue (b) in 50% sucrose, 6 M urea) and loaded on to $10 \times 0.6\text{-cm}$ cylindrical 12.5% polyacrylamide gels made in 0.1 M Tris-borate, 0.0025 M EDTA, 6.0 M urea, pH 8.3 and subjected to electrophoresis for about 3 h at 100–150 V when the bromophenol blue had migrated 9 cm. The gels were sliced into 1.5-mm segments, digested with 0.5 ml NCS and the radioactivity determined by liquid scintillation counting in a toluene-based scintillation fluid, using appropriate spill-over corrections. ●, ^3H ; ○, ^{35}S .

protein was associated with the entire heterogeneous induced ssRNA peak and not just with the 35S virus particle RNA.

We conclude from these observations that the ^{35}S -labelled material associated with the RNA extracted from ^{35}S -methionine-labelled FMDV particles is a protein of MW about 4,000 and is attached covalently. Our results are similar to those reported for poliovirus RNA (refs 5, 6). The protein linked to the RNA of poliovirus does not contain methionine⁵ but that attached to the RNA of FMDV contains at least one methionine residue. If it is assumed that the protein in FMDV contains only one residue of methionine then the proportion of radioactivity in virus particles associated with the RNA-linked protein is compatible with the presence of a single copy in each virus particle.

The absence of a cap structure or an identifiable 5' end on FMDV-RNA supports the hypothesis that, as with poliovirus RNA, a protein blocks the 5' end of the RNA. The fact that the protein does not seem to be attached to the polyC tract suggests that this homopolymer is not 5'-terminal. The association of the protein with ssRNA parallels the appearance of mature virus particles in the cells and it is tempting to speculate that the protein has a regulatory role in determining whether the RNA is incorporated into virus particles as Fernandez-Munoz and Lavi have suggested for poliovirus¹⁴. The covalently-linked protein of poliovirus RNA has been found in replicative intermediate RNA (ref. 6) and on the 5' end of negative RNA strands¹⁵, leading to the suggestion that the protein has a role in transcription, acting as a primer on the nascent RNA strands and being cleaved from the RNA destined to become polysomal mRNA. A more detailed analysis of the fate of the RNA synthesised at different times after infection and an examination of the protein linked to the replicative intermediate should help to elucidate the role of the protein in virus replication.

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Participation of DNA-dependent RNA polymerase II in replication of influenza viruses

FOR many years it has been known that inhibitors of the expression of cell DNA, such as actinomycin D, prevent the multiplication of influenza viruses; inhibitors of DNA synthesis have no such effect¹⁻³. This phenomenon is still not understood, even though from the outset the available data have suggested that cell gene transcription may be a necessary prerequisite for virus RNA transcription and

replication². The discovery that α -amanitin, a specific inhibitor of nucleoplasmic DNA-dependent RNA polymerase II (ref. 4), also specifically inhibits influenza virus replication^{5,6}, is additional support for the theory that cell gene expression is essential for virus growth. There has always been the possibility, however, that inhibition of virus growth is unrelated to the defined activities of DNA function inhibitors. It is possible, for example, that actinomycin D or α -amanitin might prevent the uptake or uncoating of influenza viruses, might inhibit directly the virion-associated transcriptase *in vivo*, or might act in some other unrecognised way. We describe here experiments in which α -amanitin was tested for its ability to inhibit the growth of influenza virus in cells containing an α -amanitin-resistant DNA-dependent RNA polymerase; in these circumstances, the virus grows normally in the presence of high concentrations of α -amanitin. This finding indicates that DNA-dependent RNA polymerase II has an essential role in the replication of influenza viruses.

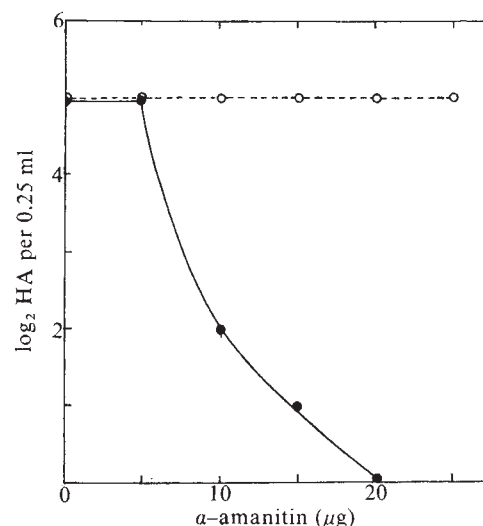


Fig. 1 Effect of increasing doses of α -amanitin on the yield of FPV from α -amanitin-sensitive, wild-type (●) and α -amanitin-resistant, mutant AMA-1 (○) CHO cells. Confluent monolayer cultures of cells were pre-incubated in medium containing α -amanitin for 1 h before infection with FPV (10 p.f.u. per cell), and α -amanitin was present in the culture medium throughout the experiment. At 24 h the amount of virus released into the medium was determined using an HA assay. Similar results were obtained when infected cells were disintegrated by Dounce homogenisation before HA determination.

The experiments were carried out on Chinese hamster ovary (CHO) cells infected with the avian influenza A virus, fowl plague (FPV). Two cell lines were kindly provided by Dr L. Siminovitch, University of Toronto, Canada, comprising the normal, α -amanitin-sensitive, wild-type CHO cell, and the mutant, α -amanitin-resistant (AMA-1) line. AMA-1 cells contain an α -amanitin-resistant DNA-dependent RNA polymerase II (refs 7-9). Cells were grown as monolayers in small plastic flasks in Dulbecco's MEM medium containing 10% foetal calf serum. For virus infection, cell sheets were washed with PBS, and allantoic fluid containing FPV was added to provide a multiplicity of infection of 10 PFU per cell. After 30 min adsorption, fresh MEM medium containing 2% foetal calf serum was added to the cells. Viral growth occurred in both cell types, and newly synthesised virus was detected either by haemagglutinin (HA) or plaque assay; maximum yields were obtained by 12 h after infection. The effects of increasing doses of α -amanitin in the culture medium on the 24-h yields of FPV from wild-type and AMA-1 cells, as determined using the HA assay, are shown in Fig. 1. Similar results were obtained when yields were measured by plaque assay. A dose-dependent inhibition of virus yield was observed only

in wild-type cells. α -Amanitin ($20 \mu\text{g ml}^{-1}$) in the culture medium produced $>95\%$ reduction in the yield of influenza virus from wild-type CHO cells, but had no effect on the yield of virus from AMA-1 cells. These results indicate that the growth and release of influenza virus in AMA-1 cells is unsusceptible to α -amanitin.

To confirm that the DNA-dependent RNA polymerase II of AMA-1 cells is resistant to α -amanitin, in both uninfected and infected cells, the effect of α -amanitin on enzyme activity in isolated cell nuclei was determined. The polymerase II activity of nuclei extracted from uninfected wild-type and AMA-1 cells, both in the presence and absence of α -amanitin ($5 \mu\text{g ml}^{-1}$) was determined using the method of Lobban and Siminovich⁸. A similar experiment was carried out using nuclei from both cell types infected 1 h previously with FPV, and the results are shown in Table 1. α -Amanitin reduced polymerase activity of both uninfected and infected wild-type CHO cells by 70–75%. The drug had no effect on polymerase levels of uninfected and infected AMA-1 cells. Virus infection seemed to increase enzyme activity by 20–30% at 1 h after infection.

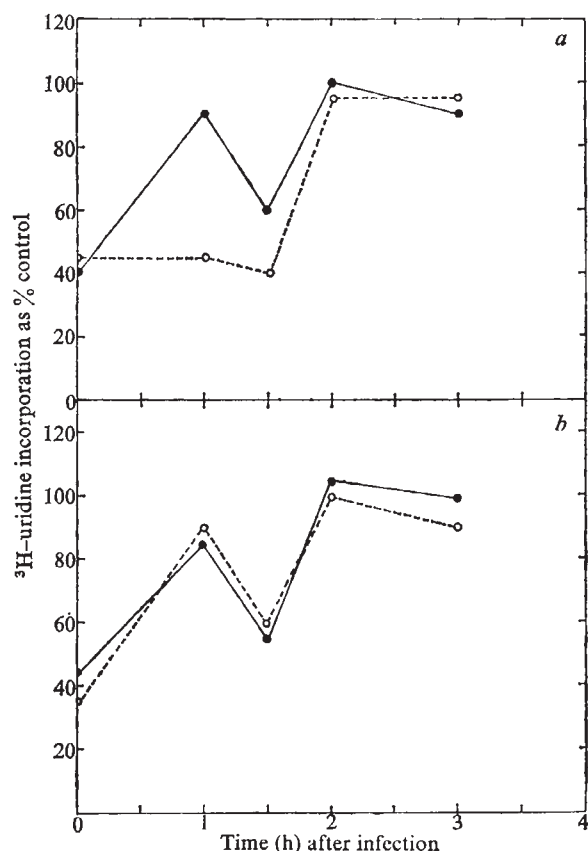


Fig. 2 Effect of α -amanitin on RNA synthesis in FPV-infected CHO cells. Confluent monolayers of wild-type and AMA-1 CHO cells were prepared in small, plastic flasks and infected with FPV at an input multiplicity of 10 PFU per cell. At various times, duplicate infected and control cultures were treated with α -amanitin $20 \mu\text{g ml}^{-1}$ for 1 h, followed by a 20-min pulse of ^3H -uridine ($5 \mu\text{Ci ml}^{-1}$, $27.6 \text{ Ci mmol}^{-1}$). Cells were then washed with TCA and the amount of insoluble radioactivity determined by scintillation counting. The results are expressed as percentage counts incorporated into

α -amanitin-treated FPV-infected CHO cells (○)
 α -amanitin treated, uninfected cells

and are compared with the percentage counts incorporated into

FPV-infected CHO cells (●)
 Uninfected cells

a, Wild-type cells; b, AMA-1 cells.

In an attempt to establish the stage of the virus replication cycle affected by α -amanitin, virus-induced RNA synthesis was determined in infected wild-type and AMA-1 cells in the presence and absence of α -amanitin. Infected cells were exposed to an inhibitory dose of α -amanitin at various times following infection, and some time later their ability to synthesise RNA was determined. This experiment was carried out by adding α -amanitin ($20 \mu\text{g ml}^{-1}$) to the culture medium for 1 h before infection, or for hourly

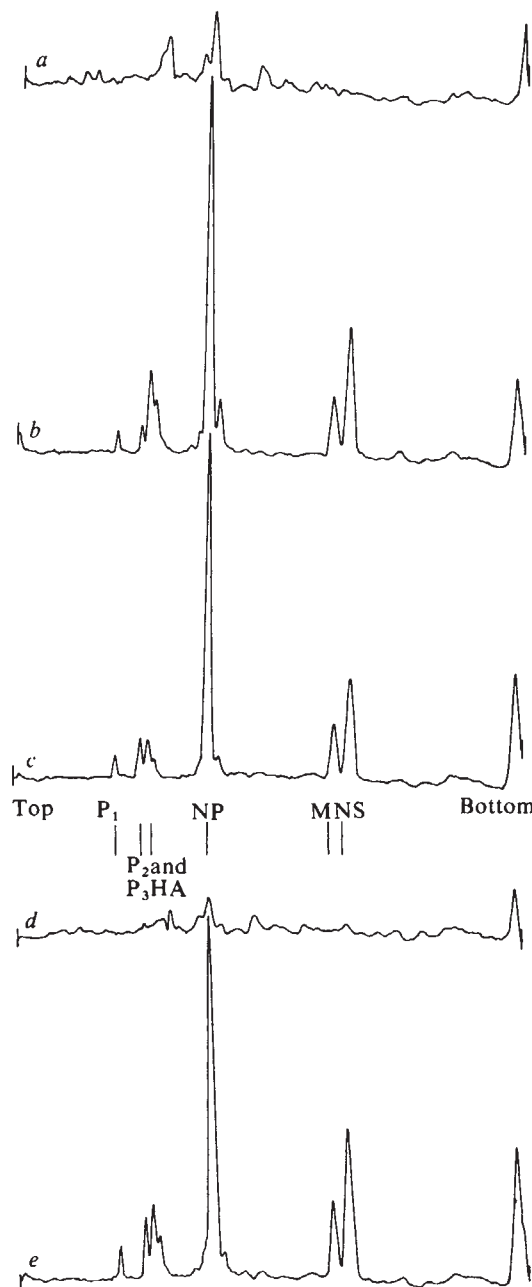


Fig. 3 Polyacrylamide slab gel electrophoresis of ^{35}S -methionine-labelled, high molecular weight polypeptides synthesised by uninfected and FPV-infected CHO cells. a, Uninfected wild-type CHO cells; b, wild-type CHO cells at $3\frac{1}{2}$ h after infection by FPV (10 PFU per cell); c, infected AMA-1 cells at $3\frac{1}{2}$ h; d, infected wild-type CHO cells at $3\frac{1}{2}$ h, α -amanitin $20 \mu\text{g ml}^{-1}$ present in the medium throughout infection; e, infected AMA-1 cells at $3\frac{1}{2}$ h, α -amanitin $20 \mu\text{g ml}^{-1}$ present in the medium throughout infection. Cells were infected or mock-infected and at 3 h received ^{35}S -methionine $5 \mu\text{Ci ml}^{-1}$ for 15 min, before being processed for electrophoresis¹². The material was loaded on to a 15% SDS-acrylamide gel, run for 16 h at 50V and processed for autoradiography. Densitometer tracings of the resulting autoradiographs are shown; the direction of migration and location of virus-induced polypeptides is indicated.

Table 1 Effect of α -amanitin on RNA polymerase II activity* of uninfected and FPV-infected CHO cells (1 h after infection)

Source of nuclei	α -Amanitin ($\mu\text{g ml}^{-1}$)	% Control (c.p.m.)†
Wild-type uninfected	0	100
	5	37
Wild-type infected	0	120
	5	30
AMA-1 uninfected	0	100
	5	103
AMA-1 infected	0	131
	5	127

*Polymerase reaction mixture is based on that described by Lobban and Siminovich⁸. In a volume of 560 μl were mixed 20 μmol Tris-HCl (pH 7.9); 0.7 μmol MnCl_2 ; 37.5 nmol each of ATP, CTP and GTP; 10 μCi ^3H -UTP at 40 Ci mmol⁻¹ specific activity; 1.1 μmol β -mercaptoethanol; 240 μmol $(\text{NH}_4)_2\text{SO}_4$, and about 150 μg of purified cell nuclei. When the assay was carried out in the presence of α -amanitin, samples were pre-incubated with 5 $\mu\text{g ml}^{-1}$ of the drug for 10 min at 37 °C. The mixture was then incubated at 30 °C for 15 min, and the reaction was terminated by addition of 1 ml $\text{Na}_4\text{P}_2\text{O}_7$ containing 0.2% bovine serum albumin. Acid-soluble radioactivity was determined by the method of Mahy and Bromley¹¹.

†Controls for this experiment were wild-type uninfected nuclei (2.36×10^6 c.p.m. ^3H -UTP mg⁻¹ nuclear protein) and AMA-1-uninfected nuclei (2.57×10^6 c.p.m. mg⁻¹).

intervals from the time of infection and then exposing the cells to a 20-min pulse of ^3H -uridine (5 $\mu\text{Ci ml}^{-1}$, 27.6 Ci mmol⁻¹). The level of TCA-insoluble radioactivity thus obtained at different times following infection in the drug-treated cells, was compared with that obtained in infected, untreated cultures. The results are shown in Fig. 2. Two peaks of virus-induced RNA synthesis (early and late) could be detected in both cell lines in the absence of α -amanitin, as has been described previously in FPV-infected primary chick cells^{6,10}. AMA-1 cells treated with α -amanitin are indistinguishable from untreated cells, but the presence of α -amanitin inhibits the early peak of virus-induced RNA synthesis in wild-type CHO cells. The results suggest that the α -amanitin-sensitive step, mediated by DNA-dependent polymerase II, occurs early in infection.

To determine whether functional virus mRNA is made in α -amanitin-treated cells, the synthesis of virus polypeptides in infected wild-type and AMA-1 cells was investigated. Both cell types were infected with FPV in the presence or absence of α -amanitin (20 $\mu\text{g ml}^{-1}$) and at 3 h after infection received ^{35}S -methionine (5 $\mu\text{Ci ml}^{-1}$) for 15 min. The cells were then processed for polyacrylamide gel electrophoresis and autoradiography according to the method described by Inglis *et al.*¹². Densitometer tracings of the resulting autoradiogram are shown in Fig. 3. α -Amanitin reduced protein synthesis in infected wild-type cells to a level below that seen in uninfected cells. Virus-induced protein synthesis in α -amanitin-treated AMA-1 cells was quantitatively and qualitatively indistinguishable from that in cells untreated with α -amanitin.

The results reported here indicate that transcription of cell DNA by DNA-dependent RNA polymerase II is essential for the replication of influenza viruses. This transcription is an early event, apparently preceding the synthesis of virus-specific mRNA.

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Dopamine selectively increases ^3H -GABA release from slices of rat substantia nigra *in vitro*

THE firing rate of the dopaminergic neurones in the substantia nigra (s. nigra) of mammalian brain is controlled largely by descending striatonigral neurones. But new evidence suggests that the activity of nigral neurones may also be modulated by local circuits within the s. nigra, involving a local dendritic release of dopamine¹. The demonstration that dopamine is present in², and released³⁻⁵ from, the dendrites of nigral neurones both *in vitro* and *in vivo* supports this concept. The precise site of action of dopamine in the s. nigra is not known, but a dopamine-sensitive adenylate cyclase has been described in this brain region⁷, apparently located presynaptically on the terminals of striatonigral afferents⁸⁻¹⁰ containing either γ -aminobutyric acid (GABA) or substance P^{11,12}. To determine whether presynaptic dopamine receptors in s. nigra are associated with substance P or GABA-containing axon terminals, we have investigated the effects of dopamine on the release of substance P and GABA from superfused slices of rat s. nigra *in vitro*. Our results show that dopamine stimulates selectively the release of GABA from nerve terminals in s. nigra.

Substantia nigra tissue (weighing approximately 6 mg, from one brain) was dissected from 0.8 mm coronal sections of fresh chilled rat brain. Initially the high affinity uptake of ^3H -GABA into 0.2 $\times$ 0.2-mm slices of s. nigra was measured, using the method of Iversen and Johnston¹³. After 10 min of incubation with ^3H -GABA (10^{-7} M) at 37 °C, a tissue : medium ratio of 72.9 ± 2.1 (mean $\pm$ s.e.m.; $n = 4$) was found, indicating that slices of s. nigra accumulated exogenous GABA avidly from the external medium.

Substance P release from superfused nigral slices was measured as described previously¹⁴. In experiments to measure GABA release, nigral tissue from two rats was pooled, incubated with ^3H -GABA, and the release of ^3H -GABA was measured in serial 2 min superfusate fractions (Fig. 1). The efflux of ^3H -GABA exhibited a rapid initial decline and after 15 min stabilised at a level which represented a loss of 0.7–0.9% of total tissue stores of ^3H -GABA per minute. Addition of depolarising concentrations of potassium chloride (11–47 mM) to the superfusing medium for 4 min produced a concentration-dependent release of ^3H -GABA. The potassium-evoked release of ^3H -GABA was abolished in media with reduced Ca^{2+} and elevated Mg^{2+} concentrations (Table 1a). Okada and Hassler¹⁵ described an electrically-evoked release of GABA from slices of rat s. nigra *in vitro*, but failed to demonstrate the calcium-dependence of this phenomenon.

Addition of dopamine (5–500 μM) to the superfusing medium for 4 min also produced a concentration-dependent increase in the rate of ^3H -GABA release (Fig. 1a, Table 1c) and this effect was completely abolished in media with reduced Ca^{2+} and elevated Mg^{2+} concentrations. The actions of dopamine in both the corpus striatum and s. nigra

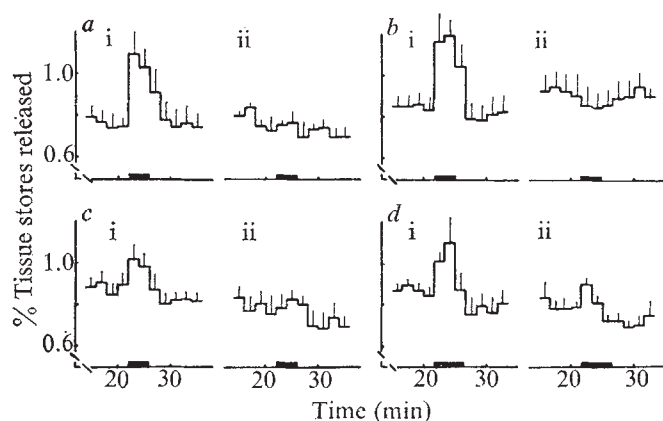


Fig. 1 Release of ^3H -GABA from rat substantia nigra. Slices of rat substantia nigra (0.2×0.2 mm) were incubated for 20 min in Krebs bicarbonate solution containing 10^{-7} M ^3H -GABA (Radiochemical Centre, Amersham; specific activity 12.6 Ci mmol^{-1}). After incubation they were transferred to a superfusion apparatus⁴ and washed at 37°C with Krebs bicarbonate (containing 30 mg l^{-1} ascorbic acid and 10 mg l^{-1} EDTA to inhibit the spontaneous breakdown of added dopamine), at a rate of 0.5 ml per min. Superfusate samples were collected at 2 min intervals and the tritium content was determined. Nigral tissue was recovered at the end of the superfusion and the total content of radioactivity measured. ^3H -GABA efflux is expressed as a fractional rate constant, which represents the amount of ^3H released per minute in each sample as a percentage of the total tissue content of ^3H -GABA. In similar experiments over 90% of the radioactivity recovered in tissue and superfusate fractions was found to represent unchanged ^3H -GABA²¹. *a*, *i*, Release of ^3H -GABA evoked by addition of 50 μM dopamine (4 min, black bar, $n = 8$). *ii*, Effect of 50 μM dopamine in medium containing 0.1 mM Ca^{2+} and 12 mM Mg^{2+} ($n = 4$). *b*, Effect of α -flupenthixol (1 μM) on dopamine-evoked ^3H -GABA release. *i*, 50 μM dopamine pulse (4 min, black bar, $n = 5$). *ii*, Dopamine 50 μM (4 min) after addition of α -flupenthixol (1 μM) from min 18 to 24 ($n = 5$). *c*, Dexamphetamine-evoked release of ^3H -GABA. *i*, Amphetamine 50 μM (4 min, black bar, $n = 6$). *ii*, Amphetamine 50 μM after addition of α -flupenthixol (1 μM) for the total period of superfusion ($n = 4$). *d*, Evoked release of ^3H -GABA by addition of dibutyryl-cyclic AMP (db cAMP). *i*, db cAMP (1.2 mM) for 6 min (black bar), $n = 4$. *ii*, db cAMP (1.2 mM) in medium containing 0.1 mM Ca^{2+} and 12 mM Mg^{2+} ($n = 4$).

are mediated by dopamine receptors linked to a specific dopamine-sensitive adenylate cyclase that is potently antagonised by neuroleptic drugs^{7,16,17}. Addition of the neuroleptics α -flupenthixol (1 μM) (Fig. 1*b*) or fluphenazine (1 μM) (Table 1*d*) to the superfusing medium completely abolished the dopamine-evoked release of ^3H -GABA. Haloperidol (1 μM) (Table 1*e*) also markedly suppressed the dopamine-evoked release. The potent dopamine agonist 4-amino-6-7-dihydroxy-1,2,3,4-tetrahydronaphthalene (ADTN) (ref. 18) (50 μM) mimicked the stimulatory effect of dopamine on ^3H -GABA release (Table 1*f*). The dopamine-evoked release of ^3H -GABA in the s. nigra thus seems to be mediated by an action on dopaminergic receptors with pharmacological specificity similar to that of receptors in other dopamine-rich brain areas.

Addition of dexamphetamine (50 μM) also caused a small but significant ($P < 0.05$) release of ^3H -GABA from slices of s. nigra (Fig. 1*c*), and this effect could be blocked by previous exposure to α -flupenthixol (1 μM). Amphetamine lacks any direct action on central nervous system (CNS) dopamine receptors¹⁸, but stimulates a release of tissue stores of dopamine in various regions of CNS including s. nigra^{2,19}. The stimulatory effect of amphetamine suggests that a release of endogenous dopamine from the dendrites of nigral neurones can stimulate a local release of GABA. A functional dendro-axonic synaptic interaction may thus exist even in superfused slices of s. nigra maintained *in vitro*.

Superfusion with the stable cyclic AMP analogue, dibutyryl-cyclic AMP (1.2 mM) also caused a calcium-dependent increase in ^3H -GABA release (Fig. 1*d*), showing that the effect of dopamine may be related to increases in intracellular cyclic AMP levels in GABA-containing nerve terminals. Minneman and Cuello (personal communication) have observed presynaptic localisation of cyclic nucleotide phosphodiesterase in the rat s. nigra, providing further evidence for the localisation of a cyclic AMP system within nerve terminals in the s. nigra.

Although there is evidence for an involvement of cyclic AMP, the cellular mechanism responsible for the dopamine-evoked release of GABA remains unknown. The calcium dependence of this phenomenon suggests a possible similarity to the mechanism involved in the normal stimulus-evoked release of transmitters. Furthermore, when nigral slices were exposed simultaneously to dopamine (50 μM) and to high potassium (11 mM) the effects on ^3H -GABA release were not additive (Table 1*b*). In parallel experiments the spontaneous efflux of substance P was found to be 28.36 ± 4.08 fmol mg^{-1} (mean $\pm$ s.e.m.; $n = 4$); addition of dopamine (50 μM) for 4 min produced no significant change in spontaneous efflux (29.84 ± 0.88 fmol mg^{-1} , $n = 4$). Similarly the potassium-evoked release of substance P (36.41 ± 4.07 fmol mg^{-1} ; $n = 3$) was unaffected by simultaneous addition of dopamine (39.26 ± 6.13 fmol mg^{-1} ; $n = 3$).

Since high affinity GABA uptake occurs into glial cells as well as neurones, it is possible that the GABA release we have described occurred partly from glial cells. But, the apparent localisation of dopamine-sensitive adenylate cyclase on striatonigral nerve terminals suggests that dopamine released within the s. nigra produces a selective stimulation of the release of GABA located within inhibitory striatonigral terminals, and this might provide the basis for a local inhibitory feedback circuit. The inhibitory effects of dopamine when applied iontophoretically into the s. nigra²⁰ may, at least in part, be mediated by such an effect of dopamine on GABA-containing axon terminals. The lack of effect of dopamine on substance P release suggests that the dopamine-sensitive adenylate cyclase in rat s. nigra may be localised predominantly on GABA-containing axon terminals.

ADTN was provided by Dr T. Inch, Chemical Defence Research Establishment, Porton Down, and substance P by Dr R. Hirschmann, Merck, Sharpe and Dohme. T.M.J.

Table 1 Effect of dopamine (DA) and high potassium (K^+) on release of ^3H -GABA from rat substantia nigra

Stimulus	Evoked release of ^3H -GABA (% of total tissue stores)
<i>a</i> K^+ 11 mM	3.78 ± 0.56 (4)
K^+ 23 mM	5.94 ± 1.02 (4)
K^+ 47 mM	9.70 ± 1.88 (4)
K^+ 11 mM; Ca^{2+} 0.1 mM, Mg^{2+} 12 mM	0.08 ± 0.12 (4)
<i>b</i> K^+ 11 mM + DA 50 μM	3.40 ± 0.62 (4)
<i>c</i> DA 5 μM	1.46 ± 0.38 (5)
DA 50 μM	2.24 ± 0.40 (8)
DA 500 μM	3.00 ± 1.16 (4)
<i>d</i> DA 50 μM + fluphenazine 1 μM	0.06 ± 0.08 (4)
<i>e</i> DA 50 μM + haloperidol 1 μM	0.42 ± 0.18 (4)
<i>f</i> ADTN 50 μM	1.52 ± 0.42 (4)

Values are means $\pm$ s.e.m. calculated as the total evoked release of ^3H -GABA in the 8-min period during and after application of a 4-min stimulus. The evoked release was calculated by subtraction of the average value for spontaneous ^3H -GABA efflux during the 6-min immediately before application of the stimulus (0.7 – 0.9% per min).

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Müller cell localisation of glutamine synthetase in rat retina

In retina as in brain, the metabolism of glutamic acid and γ -aminobutyric acid (GABA) is compartmentalised in biochemically defined pools^{1–4}. When retinas are incubated *in vitro* with radioactive glutamate and GABA, the specific activity of the glutamine produced is greater than the specific activity of the total tissue glutamate. This indicates that glutamine is formed from a small glutamate pool of higher specific activity than the total glutamate pool. To understand better the metabolic organisation of these amino acids in retina, the anatomic localisation of the small glutamate pool is essential. There is evidence that Müller cells (retinal glia) may be the site of the small glutamate pool. Autoradiographic studies indicate that mammalian Müller cells take up GABA and glutamate^{5–8}. Moreover, these cells contain succinic semialdehyde dehydrogenase⁹ and GABA-transaminase¹⁰ which are enzymes involved in the degradative metabolism of GABA. In a number of species, glutamine synthetase (GS), a key enzyme of the small glutamate pool¹¹, shows a striking increase in activity^{12–14} which parallels the morphological development of the Müller cell^{15–17}. We describe here the localisation of GS to Müller cells in rat retina by immunohistochemical techniques.

Normal adult albino rats were lightly anaesthetised with ether, and 1 ml of 1% sodium nitrite and 1,000 units of heparin sodium were injected intravenously. One minute later, rats were perfused for 15 min through the ascending aorta with 4% paraformaldehyde in 0.1 M sodium cacodylate buffer, pH 7.4, at room temperature. After perfusion, eyes were enucleated, bisected along the sagittal plane, and the vitreous body removed. Retinas were washed overnight at 4 °C in phosphate buffered saline (PBS) containing 10%

sucrose. Retinas were then rinsed for 15 min in PBS containing 12% glycerol and 4% sucrose before quick freezing in a dry ice–95% ethyl alcohol mixture. Sections (10 μ m thick) were obtained, treated with antibody to GS and processed with the indirect peroxidase-labelled antibody method^{18,19}. Details of the preparation of the anti-GS antibody, its purification and characterisation have been described previously²⁰. Briefly, immunoelectrophoretically proved monospecific antibody to sheep GS was prepared in rabbits and purified by affinity chromatography²¹. Rat and sheep GS were antigenically identical as determined by double immunodiffusion. Control sections were treated the same as experimental sections except that affinity-purified antibody to the soluble component of basement membrane (kindly provided by Dr Antonio Martinez-Hernandez) was substituted for the anti-GS antibody.

Sections of retina treated with anti-GS antibody and processed with the indirect peroxidase-labelled antibody

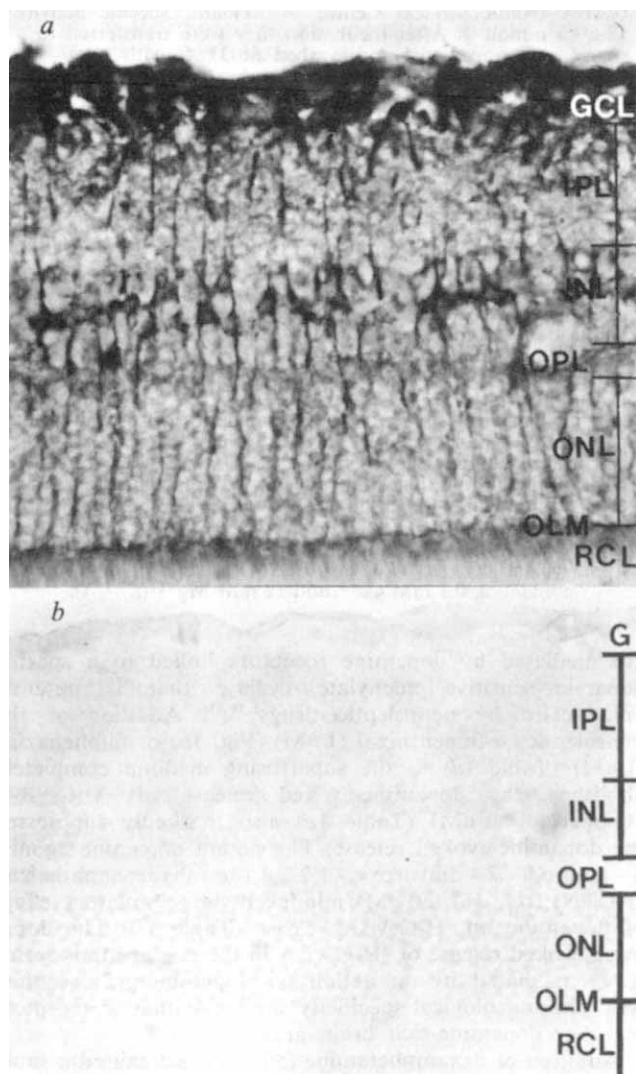


Fig. 1 *a*, Photomicrograph of rat retina cryostat section stained for glutamine synthetase by the indirect peroxidase-labelled antibody method. In the inner nuclear layer (INL), reaction product is conspicuous in Müller cell perikarya. Positive Müller cell processes extend in opposite directions from the inner nuclear layer. Densely stained areas are present in the ganglion cell layer (GCL) while small beads of reaction product form a thin line along the outer limiting membrane (OLM). RCL, rod and cone layer; ONL, outer nuclear layer; OPL, outer plexiform layer; IPL, inner plexiform layer. ($\times 300$.) *b*, Control section: rat retina treated similarly to section in Fig. 1*a* except antibody to basement membrane was substituted for GS antibody. No reaction product is present. ($\times 300$.)

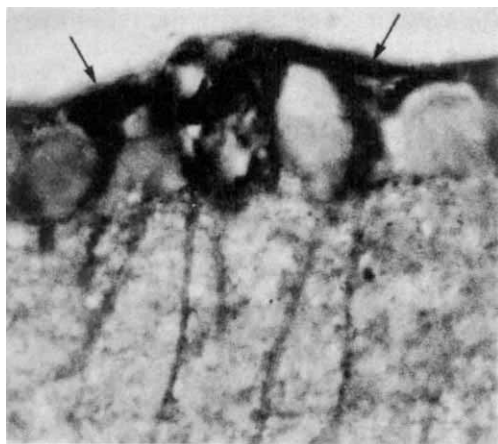


Fig. 2 Higher magnification of the ganglion cell layer demonstrates characteristic funnel-shaped Müller cell processes (arrows) containing deposits of reaction product. There is no staining in ganglion cells. ($\times 750$.)

method demonstrated that GS was confined to Müller cells (Fig. 1a). Peroxidase reaction product in the perikaryon clearly outlined the polygonal Müller cell nuclei located in the inner nuclear layer. Positive staining Müller cell processes were traced from the inner nuclear layer through the inner plexiform layer as narrow parallel columns ending in funnel-shaped deposits in the ganglion cell and nerve fibre layers (Fig. 2). Positive Müller cell processes also extended through the outer plexiform and outer nuclear layers to terminate as distinct beads of reaction product along the outer limiting membrane. The presence of GS in other glial elements could not be excluded. No reaction product was seen in photoreceptors, horizontal cells, bipolar cells, amacrine cells, or ganglion cells. Staining was not observed in retinas treated with control antibody (Fig. 1b).

This study has shown that retinal GS in the rat and therefore the small glutamate compartment associated with glutamine synthesis is localised to Müller cells, supporting the suggestion of Kennedy and co-workers⁷. Analogous to this finding is the localisation of GS to glia in rat brain²⁰. Perhaps an integral role can now be assigned to the Müller cell in the GABA-glutamate-glutamine cycle proposed to occur in retina¹. According to this concept, Müller cells take up neuronally-released glutamate and GABA, thereby inactivating and conserving these amino acids which are subsequently converted to glutamine by the action of GS. Glutamine, which is freely diffusible, then leaves the Müller cell and is available to neurones for the formation of glutamate and GABA. The localisation of GS to Müller cells thus lends firm support for a crucial role of these cells in GABA and glutamate metabolism.

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Electrical stimulation of denervated muscles reduces incorporation of methionine into the ACh receptor

ONCE a neuromuscular synapse has formed, the density of acetylcholine (ACh) receptors in the subsynaptic muscle membrane remains relatively constant, even after denervation of the muscle¹. In contrast, the density of ACh receptors in the extrajunctional muscle membrane can vary widely. Extrajunctional receptors are present at high density in developing muscle, but are reduced to low levels during maturation of muscle fibres². Their number is increased after denervation in the adult^{3,4}, and is again decreased on subsequent re-innervation of the muscle⁵. Several experiments have demonstrated that the amount and pattern of muscle activity strongly influence the density of extrajunctional ACh receptors (for review, see ref. 6), although other factors may be important as well⁶⁻⁹. Because extrajunctional receptors undergo continual turnover in the membrane¹⁰⁻¹², the effect of changes in activity on their density could result from alteration in rates of receptor synthesis or degradation. Recent experiments have shown that the reduction of receptor levels caused by stimulation in organ culture is accompanied by a decrease in the rate of receptor degradation¹³. These results imply that activity decreases the rate of receptor synthesis or its insertion into the membrane. In direct support of this idea, we report that electrical stimulation of denervated muscle reduces incorporation of ³⁵S-methionine into ACh receptors.

Left hemidiaphragms from male Sprague-Dawley rats (50-60 g) anaesthetised with ether were denervated by intrathoracic transection of the phrenic nerve. Three or four days later, denervated hemidiaphragms with ribs attached were removed from the animals, pinned out in pairs in Sylgard lined culture dishes, and incubated in 12 ml Trowells T8 medium as described previously^{10,13}. One hemidiaphragm of each pair received direct electrical stimulation by platinum electrodes recessed in the Sylgard beneath the muscle¹³, whereas the other muscle was not stimulated. Suprathreshold square pulses of 2 ms duration and alternating polarity were applied to the muscles at 40 Hz for 350 ms once every 3 s. After 17-53 h in culture, the medium was changed and the muscles incubated for 1 h with Trowells medium modified to contain a low concentration of methionine (2×10^{-6} M). ³⁵S-methionine (0.5.0 mCi; 300-500 mCi mmol⁻¹; New England Nuclear) in modified T8 medium was then added to each dish and the muscles labelled for 6-12 h.

The incorporation of ³⁵S-methionine into the ACh receptor was determined after purification by affinity chromatography on cobrotoxin-Sepharose and concanavalin A (con A-Sepharose) as depicted in Fig. 1. Diaphragms were removed, blotted dry, weighed and homogenised. A

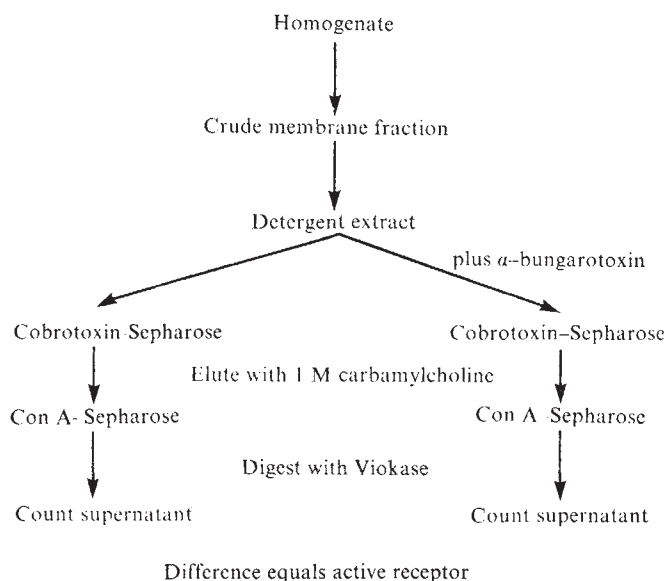


Fig. 1 Schematic outline of the procedure used to measure ^{35}S -ACh receptor. Details are provided in the text and in Table 1.

crude membrane fraction was extracted with Triton X-100 (ref. 14), and the extract was divided into two equal portions. Excess unlabelled α -bungarotoxin was added to one portion (final concentration 10^{-3} M), and both were incubated at room temperature for 15 min. The extracts were applied to parallel columns of cobrotoxin-Sepharose and eluted with carbamylcholine¹⁴. Incubation with unlabelled toxin before the column step specifically prevents adsorption of the receptor (Froehner & Hall, unpublished) and the eluate of the column receiving this extract contained only contaminating radioactivity, thus serving as a control for the paired eluate. Because the amount of contaminating radioactivity at this stage was large and variable, further purification was necessary. Since ACh receptor is a glycoprotein which binds to con A (ref. 15) additional purification was achieved by adsorbing each of the paired eluates with con A-Sepharose. Material retained on this column was digested with Viokase and the released radioactivity measured by liquid scintillation spectrometry.

Results from a typical experiment are shown in Table 1. The amount of radioactive receptor was calculated as the difference between each sample and its paired control. Ratios of radioactive receptor, corrected for wet weight, were then computed for each pair of stimulated and unstimulated muscles. Control experiments established that receptor from both stimulated and unstimulated muscles was recovered equally well from the cobrotoxin-Sepharose and was quantitatively retained on con A-Sepharose.

Results of ten experiments in which muscles were stimulated for 1–3 d are shown in Table 2. Stimulation of the muscles reduced the total amount of ACh receptor present in muscle extracts and reduced incorporation of ^{35}S -methionine into the receptor. Both the reduction in total amount of receptor and in the extent of incorporation were greater with longer periods of stimulation. Complete inhibition of synthesis was not observed even after 3 d. The residual synthesis probably reflects inadequate stimulation of some fibres¹³, but could be due to suboptimal parameters of stimulation or to the presence or absence of some factor unrelated to activity.

Because the phasic pattern of stimulation used in these experiments was different from the stimulation (2–10 Hz) used in earlier experiments¹³, we repeated our previous comparison of receptor degradation in stimulated and unstimulated muscles. When muscles were stimulated

physically, the rate of receptor degradation ($t_{1/2} = 12.08 \pm 0.8$ h, $n = 3$) was not significantly different from that found without stimulation ($t_{1/2} = 11.8 \pm 0.9$ h, $n = 3$). These results are in agreement with our previous conclusions that the decrease in ACh receptor levels in stimulated muscle is not caused by an increased rate of breakdown.

Incorporation of ^{35}S -methionine into richloroacetic acid (TCA)-precipitable material in the Triton X-100 extracts from stimulated muscles was identical to that found in control muscles ($105 \pm 9\%$, mean $\pm$ s.e.m., $n = 10$). Thus it is unlikely that differences in specific activity of intracellular methionine could account for the difference we have observed. Autoradiographs of two-dimensional gels¹⁶ of the labelled extracts from stimulated and unstimulated muscles were almost identical, differing only by a few proteins. Since neither the major patterns of protein synthesis nor the total amount of isotope incorporated is changed by stimulation, the effect on ^{35}S -methionine incorporation into receptor must be relatively specific.

The amount and pattern of muscle activity is known to affect several other properties of muscle fibres besides extrajunctional ACh receptor level. These include the species of myosin light chains made by the fibres¹⁷, the rate of synthesis of myosin heavy chain¹⁸, the amount of acetylcholinesterase activity in the muscle¹⁹ and several physiological properties of the muscle, the biochemical basis of which is not known⁶. Many of these changes seem to be controlled at the level of gene expression^{20,21}. The mechanism by which muscle activity mediates these effects is a major question for future investigation.

Previous experiments have shown that incorporation of

Table 1 Determination of ^{35}S -receptor

	Stimulated c.p.m. per mg wet weight		Unstimulated c.p.m. per mg wet weight	
Homogenate (TCA-precipitable)	4.4×10^5		2.8×10^5	
Triton X-100 extract (TCA-precipitable)	1.5×10^5		1.1×10^5	
	– α -BUTX + α -BUTX		– α -BUTX + α -BUTX	
Con A-Sepharose retained	8.2	6.2	28.6	11.4
Difference	2.0		17.2	

α -BUTX, α -Bungarotoxin.

Two left hemidiaphragms that had been denervated 4 d earlier were cultured for 3 d and labelled with 1 mCi ^{35}S -methionine for the final 12 h. The stimulated and unstimulated muscles weighed 66 and 78 mg, respectively. Each was homogenised in 1 ml 50 mM Tris-HCl, pH 7.4, 50 mM NaCl with 1 mM EDTA, 1 mM EGTA, 0.4 mM phenylmethylsulphonyl fluoride (PMSF), 20 U ml⁻¹ Trasylol (Sigma) and 0.1 μg ml⁻¹ Pepstatin. Homogenates were diluted to 5 ml with buffer and centrifuged at 48,000 r.p.m. for 45 min in a Spinco SW 50.1 rotor. The pellet was resuspended in 500 μl of the above buffer. Triton X-100 was added to a final concentration of 3% and extraction carried out on ice for 1 h. The extract was centrifuged at 20,000g for 1 h and the resultant supernatants were each divided into two equal portions. To one aliquot of extract from each muscle was added α -bungarotoxin (final concentration 10^{-5} M α -BUTX); followed by incubation of each portion for 15 min at room temperature. Four 0.3-ml cobrotoxin-Sepharose¹⁴ (400 μg cobrotoxin per ml bed volume) columns were poured and washed consecutively with 50 mM Tris-HCl, pH 7.4, 1% Triton X-100 (TT) containing 1 M NaCl and with 50 mM Tris-HCl, pH 7.4, 50 mM NaCl and 1% Triton X-100 (TNT). The pre-incubated extracts were then adsorbed on to the columns at 4 °C, and the columns washed sequentially with TNT (5 ml), TT+1 M NaCl (10 ml) and TNT (5 ml). In each case no radioactivity was detectable in the final wash. ACh receptor was then eluted with several column volumes of TNT which contained 1 M carbamylcholine chloride, pH 8.6, and 0.4 mM PMSF, 20 U ml⁻¹ Trasylol and 0.1 μg ml⁻¹ Pepstatin. Duplicate 25- μl aliquots from 1-ml eluates were counted and the remainder adsorbed to pre-washed 0.5 ml con A-Sepharose columns (2 mg con A ml⁻¹ bed volume). After the columns were washed with TNT to remove non-adsorbed radioactivity, the resin was mixed with 0.5 ml 3 mg ml⁻¹ Viokase in TNT and incubated overnight at 37 °C. The con A-Sepharose was removed from suspension by centrifugation and the supernatant radioactivity determined.

Table 2 Effect of stimulation on ACh receptor synthesis

Duration of culture (h)	Duration of labelling (h)	Total ACh receptor stimulated/unstimulated	³⁵ S-ACh receptor stimulated/unstimulated	n
24*	6	81 ± 4%	42 ± 7%	4
51†	6	39 ± 10%‡	23 ± 7%	3
66†	12	28 ± 8%‡	14 ± 2%§	3

*Denervated 3 d before culture.

†Denervated 4 d before culture.

‡Significantly different from 24 h stimulation $P < 0.005$.§Significantly different from 24 h stimulation $P < 0.05$.

Effect of stimulation on ACh receptor synthesis. Culture duration is indicated. ³⁵S-methionine was present in the culture for the time shown just before termination of the experiment. Total ACh receptor was determined by a DEAE filter-binding assay of ¹²⁵I- α -bungarotoxin as described previously¹⁴. ³⁵S-ACh receptor determined as indicated in Table 1. Values expressed as the mean $\pm$ s.e.m. n equals the number of muscle pairs. In two cases (24 h and 66 h of culture, respectively) no difference (100%; 102%) in incorporation was observed between stimulated and control muscles. Total ACh receptor was reduced in both cases; therefore, either stimulation was effective only during part of the culture period before labelling, or recovery of radioactivity was not the same for control and stimulated muscles. Because of the large, statistically significant variation of these values from the others, results from these two experiments have been excluded from the data in Table 2. Their inclusion, however, would not affect the general conclusions to be drawn.

³⁵S-methionine into the ACh receptor can be detected in denervated muscles only after accumulation of extra-junctional receptor begins²². These results along with those reported here show that changes in incorporation of ³⁵S-methionine into ACh receptor parallel changes in overall receptor density and suggest that extrajunctional receptor levels are controlled by regulation either of synthesis or of some other step before appearance of the receptor in the external membrane.

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High conductance in an epithelial membrane not due to extracellular shunting

THERE is conclusive evidence for a cellular and extracellular route for ions across epithelial tissues¹. The extracellular pathway is constituted by the tight junctions and the lateral inter-cellular space. Epithelia have been classified as 'tight' or 'leaky' depending on the ratio of the conductances of both pathways (that is, in a tight epithelium the ratio: $g_{\text{shunt}}/g_{\text{cell}} \leq 1$). Moreover, tight epithelia have transmural resistances and electrical potentials far greater than those observed in leaky epithelia. We report here that the epithelium of rabbit salivary duct does not confine to this rule since it combines several characteristics of tight epithelia with resistance values as low as in proximal tubules of the kidney, an extremely leaky epithelium. Permeability studies with polar non-electrolytes support the conclusion that the high conductance is not due to extracellular shunting. Our study implies that the magnitude of the epithelial resistance does not determine whether an epithelium is tight or leaky.

Main ducts of rabbit salivary glands were dissected and mounted in a perfusion chamber as before². Transductal resistance was measured by a direct method. We developed a combined current passing and potential sensing electrode small enough to fit into the lumen of the duct. Details of the electrode are published elsewhere³. Ductal permeability to non-electrolytes was determined by conventional isotope techniques. Unidirectional fluxes of labelled compounds were measured from serosa to mucosa. Ringer solutions contained (in mM): Na, 145; K, 4; Mg, 1; HCO₃, 25; acetate, 10; pyruvate, 10; pH, 7.4. The other anions were Cl, 100 or SO₄, 50 respectively. Sulphate solutions have mannitol to restore isotonicity and also 2 mM Cl for proper functioning of the Ag/AgCl electrode³. The serosal solution contained in addition 6 mM glucose and protein (Haemacel, 30 g l⁻¹) to keep ductal transport optimal².

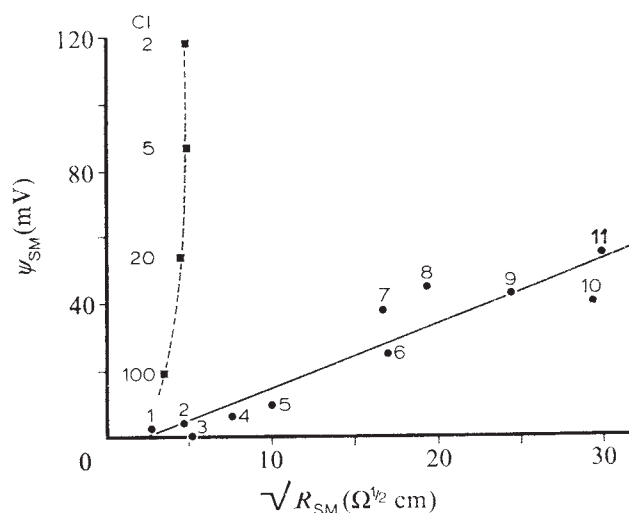


Fig. 1 Relationship between transepithelial potential (ψ_{SM}) and the square root of resistance in various epithelia (straight line). (1), Proximal tubule, rabbit (ref. 4); (2), ileum, rabbit (ref. 5); (3), gall bladder, rabbit (ref. 6); (4), gall bladder, human (ref. 7); (5), early distal tubule, rat (ref. 8); (6), colon, rabbit (ref. 9); (7), gastric mucosa, lizard (ref. 10); (8), late distal tubule, rat (ref. 11); (9), late distal tubule, dog (ref. 12); (10), collecting duct, rabbit (ref. 13); (11), collecting duct, hamster (ref. 14). The dashed line gives values of ψ_{SM} and $\sqrt{R_{\text{SM}}}$ measured for rabbit main duct in solutions with varying Cl-concentrations. Solutions were symmetrical and the number indicate Cl concentration. In low-Cl solutions current pulses induced strong polarisation effects and voltage responses were extrapolated to dV/dI ($t \rightarrow 0$). In high-Cl solutions polarisation effects were minimal. Our resistance values in high Cl solutions agree well with values obtained from applying cable theory as reported by Fromter *et al.*²⁴. $y = 1.89x - 4.2$; $r = 0.94$.

In Fig. 1 electrical potentials (Ψ_{SM}) are plotted against the square root of resistance values ($\sqrt{R_{SM}}$) reported for various epithelial tissues. The correlation between these parameters is highly significant, indicating that the smaller the extracellular shunt conductance the higher the values for Ψ_{SM} and R_{SM} . The dashed line in Fig. 1 gives that same relation for salivary duct epithelium, which shows a totally different behaviour. Besides the fact that Cl ions have a strong short circuiting effect on the potential we observed in SO_4 solutions potentials up to 140 mV combined with resistance values as low as $10 \Omega \text{ cm}^2$. Potentials of the same magnitude have been reported only for tight epithelia with resistance values three orders of magnitude greater¹⁶. Cl ions have also a short-circuiting effect on frog skin potentials, although less pronounced¹⁵.

In Fig. 2 values of Ψ_{SM} and $\sqrt{R_{SM}}$ are plotted as a variation among 23 main ducts, measured immediately after mounting in SO_4 solutions. There is a statistically significant inverse relationship between these two parameters. A similar inverse relationship has been reported for urinary bladders, however,

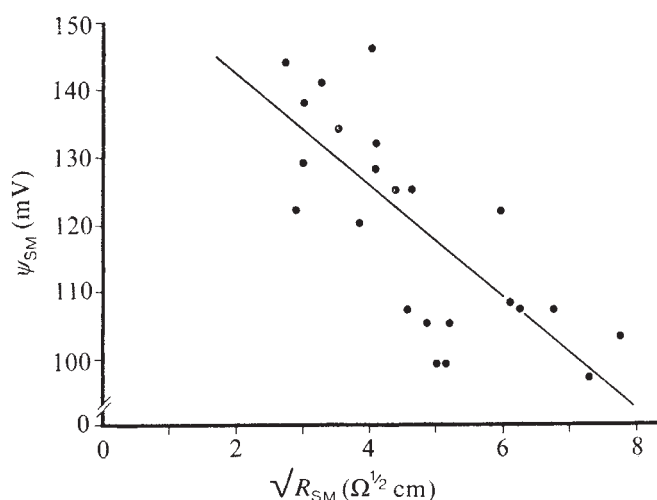


Fig. 2 Inverse relationship between potential difference (Ψ_{SM}) and resistance ($\sqrt{R_{SM}}$) as a variation among 23 main ducts measured in symmetrical SO_4 solutions with 2 mM Cl. $y = -8.25x + 158.3$; $r = 0.75$.

only when special measures were taken to exclude edge damage effects^{16,17}. Evidently an inverse relationship will not occur when a 'passive' extracellular shunt determines the variation in the transepithelial resistance. The phenomenon can be explained when the rate of active Na-transport is regulated by the Na-conductance of the luminal cell membrane¹⁶. A greater Na conductance would then evoke higher rates of Na pumping in the contraluminal membrane. Figures 1 and 2 show clearly that the main duct combines tight epithelial properties with resistance values normally observed in very leaky epithelia. The results given in Table 1 support this ambiguity. Permeabilities to polar non-electrolytes in the main duct are of the same order as those in the toad urinary bladder. The apparent impermeability to inulin suggests that a significant paracellular shunt for non-electrolytes is absent. On the other hand, a greater P_{Cl} value reflects the low resistance of the duct.

Three more arguments are in favour of ranking ductal epithelium as tight despite the low resistance. The natriuretic drug amiloride inhibited net Na-transport in salivary duct completely when it was applied to the mucosal solution (10^{-4} M). In SO_4 -solutions ductal resistance increased from 22.3 ± 1.8 to $91.4 \pm 12.6 \Omega \text{ cm}^2$ ($n = 7$) after addition of amiloride. In Cl-solutions the increase was less pronounced (from 6.6 ± 0.4 to $14.1 \pm 0.7 \Omega \text{ cm}^2$, $n = 4$). The relative increase in resistance is comparable with amiloride treatment of frog skin¹⁵.

In Cl-solutions we measured a net Na-absorption rate

Table 1 Permeability of main duct, urinary bladder and gallbladder to polar non-electrolytes

	Rabbit submaxillary main duct	Toad urinary bladder	Rabbit gall bladder (ref. 19)
Solute	($10^{-7} \text{ cm s}^{-1}$)	($10^{-7} \text{ cm s}^{-1}$)	($10^{-7} \text{ cm s}^{-1}$)
Urea	56	14 (ref. 18)	890
Sucrose	6.5	5.1 (ref. 17)	76
Inulin	n.d.	n.d. (ref. 17)	29
Cl ⁻	550 (ref. 21)	13 (ref. 17)	—

The tubular perfusion rate was $12.5 \mu\text{l min}^{-1}$. P values of main duct are quoted as the mean estimated from five estimates (n.d., not detectable). P_{Cl} was determined in ducts with open circuit. P_{Cl} was independent of the electrical potential (for detailed discussion see ref. 21).

of $587 \text{ nEq min}^{-1} \text{ cm}^{-2}$, comparable with $683 \text{ nEq min}^{-1} \text{ cm}^{-2}$ reported by Martin *et al.*²², corresponding with $0.94\text{--}1.10 \text{ mA cm}^{-2}$. Potential and resistance values in this condition were $12.3 \pm 2.4 \text{ mV}$ and $10.8 \pm 1.9 \Omega \text{ cm}^2$ ($n = 9$). From these values a short-circuit current (SCC) of 1.14 mA cm^{-2} can be calculated which equals net Na-transport. The SCC is only equal to net Na-transport in tight epithelia^{17,23}. Furthermore, salivary duct epithelium responds asymmetrical to cation gradients contrasting with symmetrical responses in leaky epithelia²⁴.

Interestingly, the resistance of cell membranes can be as low as $10 \Omega \text{ cm}^2$ —we found no reason to ascribe this low resistance to an extracellular shunt pathway.

From the unusual combination of low resistance and electrical properties of tight epithelia we have to conclude that the only criterion whether an epithelium is 'tight' or 'leaky' is the conductance ratio of the cellular and extracellular route since the electrical resistance alone is not sufficient to discriminate between the two classes of epithelia.

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Fluorescent probe detects redistribution of cell calcium during stimulus-secretion coupling

STIMULUS-secretion coupling in pancreatic acinar cells is believed to be mediated by a rise in intracellular Ca^{2+} activity¹⁻⁵. Based on the observation that physiological secretagogues trigger a rapid increase of Ca^{2+} efflux^{3,4} and stimulate enzyme release even in the absence of extracellular calcium^{2,4,5}, it has been proposed that stimulus-secretion coupling involves a redistribution of intracellular calcium^{3,5,6}. Since most calcium in pancreatic acinar cells is bound to membranes or is sequestered in mitochondria⁷, we have attempted to monitor these calcium stores during secretion by using the fluorescent probe chlorotetracycline (CTC). This probe forms highly fluorescent complexes with Ca^{2+} and Mg^{2+} in the presence of biological membranes or detergent micelles^{8,9} and has been successfully used in isolated mitochondria^{10,11} and sarcoplasmic reticulum vesicles¹² to monitor calcium movements induced by substrates and inhibitors. In this report, we show that fluorescence of CTC associated with pancreatic acinar cell membranes decreases rapidly on stimulation of enzyme secretion and that this intensity change is preferential for CTC complexed to calcium.

Enzymatically-dissociated pancreatic acini were pre-incubated 60 min in 10^{-4} M CTC, then resuspended in buffer containing no CTC and placed in a fluorometer cuvette. The fluorescence intensity of these acini decreased with time, the decrease usually being faster in the first 5 min of resuspension and slower thereafter (Fig. 1a). Application of the cholinergic agonist, bethanechol, during the slower phase (Fig. 1b) elicited a marked loss of fluorescence occurring largely in the first 6 min of

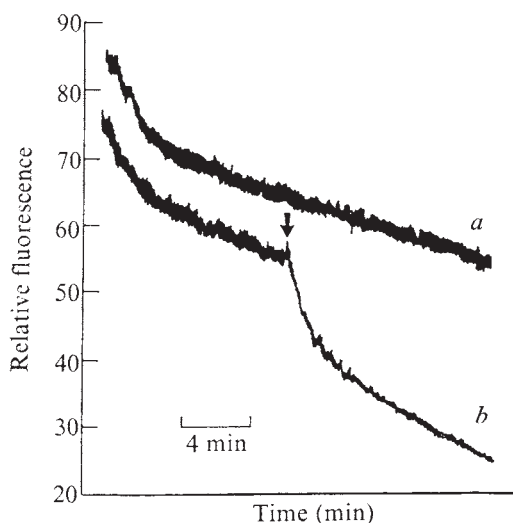


Fig. 1a, Effect of bethanechol on the fluorescence of dissociated acini loaded with chlorotetracycline (CTC). Enzymatically dissociated acini were prepared from the pancreata of 8 to 12 fasted male Swiss white mice by the method of Amsterdam and Jamieson¹¹ modified to exclude the Ca^{2+} chelation step¹⁵. Acini were preincubated for 60 min in a Tris-buffered Ringer (TR)² containing 1% bovine serum albumin (BSA) and 10^{-4} M CTC. Acini were then washed and resuspended in 4 ml of TR containing no BSA or CTC and the suspension placed in a cuvette in an Aminco-Bowman spectrofluorometer having a band pass of 15 nm and modified so as to allow continual stirring and temperature control at 37 °C. Excitation of CTC fluorescence was at 400 nm and emission monitored at 525 nm. **b**, Bethanechol (final concn 10^{-4} M) was added at the arrow and resulted in a rapid loss of fluorescence intensity. This experiment was representative of 22 similar experiments.

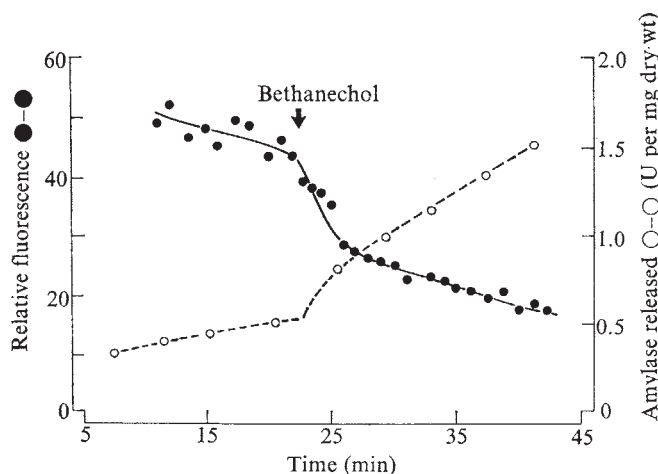


Fig. 2 CTC fluorescence of and amylase release from dissociated pancreatic acini. Acini were loaded with CTC as described in Fig. 1, then stirred periodically in a fluorometer cuvette maintained at 37 °C. Fluorescence was monitored as in Fig. 1. Amylase released into the medium was measured by removing 0.2 ml aliquots of the suspension at the designated times, centrifuging at 8000g for 20 s, and assaying amylase in the supernatant by the method of Rinderknecht *et al.*¹⁶. Amylase activity is expressed in international units per mg dry wt of acini; the cuvette contained 1.47 mg dry wt of acini ml⁻¹. Addition of bethanechol at the arrow (final concn = 1.25×10^{-4} M) elicited a rapid decrease in fluorescence and a 240% increase in the rate of amylase release. These results are representative of six similar experiments.

secretagogue action. After this time, fluorescence intensity continued to decrease at a rate similar to that before bethanechol addition. The secretagogue-induced fluorescence decrease and a stimulation of amylase release into the medium could be measured simultaneously on acini incubated in a cuvette as shown in Fig. 2. As observed previously, an increased loss of fluorescence occurred in the 5 min following bethanechol addition. At the same time, the rate of amylase release measured 5–15 min after the addition of bethanechol was increased by 240% over the control rate measured in the 15 min before adding bethanechol. This suggests that the CTC present in these acini did not interfere with normal stimulation of secretion, a result supported by experiments in which acini pre-incubated 60 min with and without 10^{-4} M CTC released identical amounts of amylase in response to bethanechol (not shown).

Two observations indicated that the fluorescence signal from CTC-loaded acini was emitted largely from probe molecules incorporated into intracellular membranes which bind Ca^{2+} and Mg^{2+} . First, fluorescence microscopy showed that CTC fluorescence in acinar cells had a distribution similar to that of intracellular membranes¹³. Second, the fluorescence excitation spectrum of CTC in acinar cells was like that of CTC complexed with divalent cations in an amphipathic environment ($\lambda_{\text{max}} = 386\text{--}400$ nm) but unlike that of free CTC ($\lambda_{\text{max}} = 382$ nm) or of CTC complexed with divalent cations in aqueous solutions. In this environment, CTC fluorescence could be decreased by dissociation of its divalent cation complexes or by subsequent partitioning of the probe out of the membrane. We have detected, in fact, an increased exit of CTC from pancreatic acini during stimulation as well as the rapid decrease in fluorescence. This exit was not due to loss of CTC associated with contents of secretory granules since doses of bethanechol that elicited maximal amylase release produced fluorescence changes that were only 30% of maximum. Furthermore, fluorescence microscopy showed that secretory granule contents do not fluoresce¹³. Rather, studies with isolated mitochondria have indicated that complex dissociation and probe

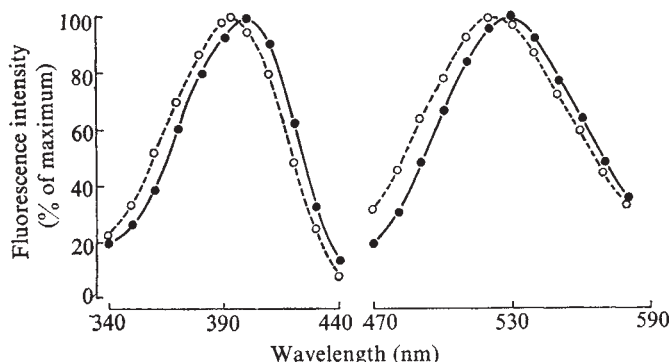


Fig. 3 Excitation and emission spectra of acinar cell-associated CTC immediately before (●) and 9 min after (○) bethanechol stimulation. Pancreatic acini were loaded with CTC, and their fluorescence monitored as described in Fig. 1. Spectra were corrected by subtracting the light scattering of an acini suspension not treated with CTC, then normalised to more clearly demonstrate the spectral shifts. Fluorescence intensity of maxima before and after stimulation were 70 and 45 units respectively. These spectral shifts were observed in each of five experiments with bethanechol and in four experiments using caerulein.

exit both occur when divalent cations dissociate from membranes containing CTC¹⁰. Thus, a reasonable explanation for the bethanechol-induced decrease in fluorescence is that Ca^{2+} or Mg^{2+} is released from some intracellular membrane.

Since Ca^{2+} and Mg^{2+} complexes of CTC can be distinguished by their excitation and emission spectra, we decided to record these spectra from CTC-loaded acini before and after stimulation. Normalised spectra in Fig. 3 show that before stimulation excitation and emission maxima were at 399 nm and 526 nm respectively. After stimulation these maxima were shifted to 393 nm and 520 nm. For comparison, model studies were done using 10^{-5} M CTC in 90% methanol-10% 10 mM Tris-HCl, pH 7.4, an amphipathic solvent in which the Ca^{2+} and Mg^{2+} complexes of CTC exhibit spectra similar to those in membrane environments¹⁰. In this solvent, Ca^{2+} -CTC exhibited excitation and emission maxima at 398 nm and 529 nm respectively, while these maxima for Mg^{2+} -CTC were at 386 nm and 519 nm. Thus, spectra from CTC-loaded acini indicated that CTC was complexed largely with Ca^{2+} before stimulation and largely with Mg^{2+} after stimulation. We concluded that the bethanechol-induced fluorescence decrease involved a preferential loss of signal from CTC complexed with Ca^{2+} in a membrane environment.

An intensity decrease accompanied by similar spectral shifts was also observed during stimulation by caerulein, a polypeptide containing the active site of CCK-PZ. The decrease in CTC fluorescence induced by bethanechol was blocked by atropine whereas that induced by caerulein was not. The fluorescence decrease in response to either secretagogue could be observed in the absence of extracellular calcium which was also the case for amylase release².

In control experiments, acini exhibited no changes in fluorescence intensity on stimulation when loaded with 1-anilino-8-naphthalenesulphonate (ANS) or oxytetracycline, fluorescence probes that are not sensitive to membrane-bound divalent cations.

In view of the known sensitivity of chlorotetracycline fluorescence to membrane-bound divalent cations⁸⁻¹², these data are direct evidence for a release of calcium from intracellular membranes during stimulus-secretion coupling in pancreatic acinar cells. We are currently using chlorotetracycline to study the site and mechanism of this calcium release.

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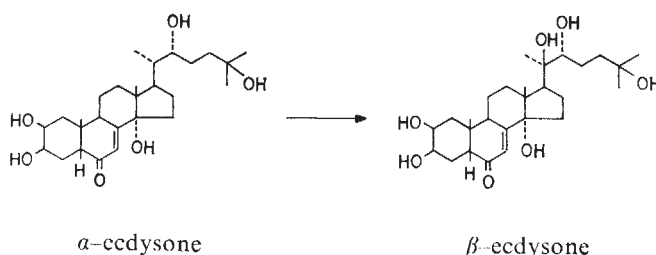
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Evidence for an α -ecdysone cytochrome P-450 mixed function oxidase in insect fat body mitochondria

It is now evident that α -ecdysone, a polyhydroxylated steroid, is synthesised and secreted by the insect prothoracic (ecdysial) glands¹⁻³. β -ecdysone (20-hydroxy- α -ecdysone) seems, however, to have the major role in eliciting moulting during the post-embryonic development of insects⁴. If α -ecdysone functions as a pro(pre)-hormone as has been suggested^{1,4}, then it must undergo hydroxylation at C-20 of the side chain to form the moulting hormone, β -ecdysone. This conversion of α - to β -ecdysone is carried out by a hydroxylase which is located in tissues peripheral to the prothoracic glands^{1,5,6}. Little is known about the subcellular distribution of this enzyme complex and there is virtually no information regarding the type of enzyme complex responsible for the oxygenation of steroids in insects in general. Using the vertebrate mixed function oxidase system as a model, we have investigated the subcellular distribution and enzymatic characteristics of the insect C-20 hydroxylase in last instar larvae of the tobacco hornworm, *Manduca sexta*.

In vertebrates, steroid hormone hydroxylations are catalysed by hydroxylase systems possessing a terminal cytochrome P-450 (refs 7 and 8). These enzyme complexes, termed mixed function oxidases or mono-oxygenases, incorporate one atom of molecular oxygen into substrate while reducing the other

Fig. 1 Stoichiometry of a cytochrome P-450 mixed function oxidase type reaction and the C-20 hydroxylation of α - to β -ecdysone.



atom to water (Fig. 1). These P-450 mixed function oxidases are located in mitochondria and/or microsomes, are NADPH dependent and exhibit a characteristic CO difference spectrum absorption maximum at A_{450} . Since the C-20 hydroxylation of α -ecdysone in insects has characteristics in common with the P-450 mixed function oxidases mediating the hydroxylation of vertebrate steroid hormones (Fig. 1), it is reasonable to suggest that oxygenation of α -ecdysone is carried out by a similar enzyme complex.

C-20 hydroxylase activity was examined in whole tissues and homogenates incubated *in vitro* with 23,24- ^3H - α -ecdysone (58 Ci mM^{-1}) to determine relative tissue hydroxylase activity. For both whole tissues and homogenates, the highest hydroxylase activity was associated with fat body, with lesser activity exhibited by Malpighian tubules, body wall, midgut, and perhaps salivary glands. C-20 hydroxylation of α -ecdysone did not occur in prothoracic glands, nerve cord, muscle, wing disks and haemolymph. Due to the high hydroxylase activity of the fat body, its abundance relative to other tissues, its accessibility and role as the major metabolic organ in insects, it was the tissue of choice for fractionation studies and biochemical characterisation of the C-20 hydroxylase.

Subcellular fractions of fat body homogenates were obtained by differential centrifugation and the C-20 hydroxylase activity determined (Table 1). The majority of the enzyme activity was associated with the mitochondrial fraction (64%), with reduced levels in the nuclear and microsomal fractions and none in the postmicrosomal supernatant. When the subcellular fractions were reconstituted, the C-20 hydroxylase activity for those pooled fractions approximated the sum of the activity for the individual fractions. This suggested that the enzyme activity associated with the nuclear and post-mitochondrial fractions may be a consequence of mitochondrial contamination. To verify this, the structural components of the subcellular fractions were examined by electron microscopy. The principal organelle observed in each fraction corresponded to what was expected, and the relative distribution of mitochondria correlated with the distribution of C-20 hydroxylase activity in the respective fractions. Mitochondrial and post-mitochondrial marker enzyme activities, cytochrome oxidase⁹ and glucose-6-phosphatase¹⁰ (possibly trehalose-6-phosphatase in insects¹¹), respectively, were then monitored in each fraction

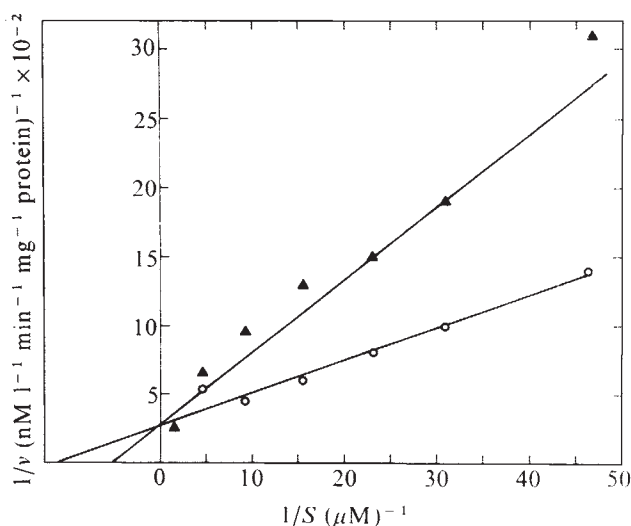


Fig. 2 Lineweaver-Burk plot for metyrapone inhibition of the fat body mitochondrial C-20 hydroxylase. The K_i for metyrapone (▲) is 3.9×10^{-6} M with a correlation coefficient of 0.997. The K_m for α -ecdysone is $1.6 \pm 0.7 \times 10^{-7}$ M (mean $\pm$ s.d. of 8 determinations). The K_m for α -ecdysone (○) in determining the metyrapone K_i was 9.0×10^{-8} M.

and their distribution compared with that for the C-20 hydroxylase (Table 1). The percentage distribution of activity for the mitochondrial marker enzyme was consistent with that observed for the C-20 hydroxylase, whereas the post-mitochondrial enzyme activity was associated with the lowest C-20 hydroxylase activity. Therefore, on the basis of these data, it seems that the α -ecdysone C-20 hydroxylase is a mitochondrial enzyme and that the activity of the nuclear and microsomal fractions is due to mitochondrial contamination. This agrees with recent preliminary reports in which mitochondrial fractions from the midgut of *M. sexta*¹² and Malpighian tubules of the locust

Table 1 Distribution of α -ecdysone C-20 hydroxylase, cytochrome oxidase and glucose-6-phosphatase activities in subcellular fractions of *M. sexta* larval fat body

Fraction	β -Ecdysone generated ng	Relative activity	Relative activity Cytochrome oxidase	Relative activity Glucose-6-phosphatase
Whole homogenate	0.32	1.0	1.0	1.0
Nuclear (N)	0.07	0.23	0.28	0.12
Mitochondrial (M)	0.21	0.64	0.58	0.15
Postmitochondrial supernatant (PMS)	0.04	0.13	0.14	0.73
microsomal	0.04	0.13		0.34
postmicrosomal supernatant	0.0	0.0		0.39
N plus M	0.28	0.87		
N plus PMS	0.10	0.32		
M plus PMS	0.22	0.69		
N plus M plus PMS	0.31	0.97		

Fat body from day 1 fifth instar *M. sexta* larvae was homogenised (50 mg ml^{-1}) in 0.05 M $\text{NaH}_2\text{PO}_4 \cdot \text{Na}_2\text{HPO}_4$ buffer (pH 7.5) containing 0.25 M sucrose at 0 °C. Subcellular fractions were obtained by differential centrifugation at -5 °C: nuclear, 800g for 10 min; mitochondrial, 12,000g for 10 min; microsomal, 105,000g for 1 h. Pellets were washed once and resuspended in the same buffer to the original concentration of 50 mg tissue equivalents ml^{-1} . For the C-20 hydroxylase assay, 0.05 ml subcellular fraction was incubated with 0.05 ml (0.05 M $\text{NaH}_2\text{PO}_4 \cdot \text{Na}_2\text{HPO}_4$ buffer (pH 7.5) containing 1.5×10^5 d.p.m. 23,24- ^3H - α -ecdysone (58 Ci mM^{-1} , 1.2×10^{-8} M) and 37.5 μg NADPH (4.0×10^{-4} M) for 30 min at 30 °C. All assays were run in duplicate. Ethanol (2.0 ml) was added to terminate the reactions. The efficiency of separation was greater than $94 \pm 2.5\%$ that obtained using high-pressure liquid chromatography³. Cytochrome oxidase⁹ and glucose-6-phosphatase¹⁰ activities, as well as protein concentrations¹⁸, were determined as described previously. Glucose-6-phosphatase has not been demonstrated to be a microsomal marker enzyme in insects but has been shown to be associated with postmitochondrial fractions, and may actually be trehalose-6-phosphatase, which is a post-mitochondrial enzyme in insects¹¹. Since trace amounts of substrate were used for the C-20 hydroxylase assay, potential compartmentalisation of endogenous α -ecdysone into selected subcellular fractions could have reduced the apparent hydroxylase activity in these fractions. Therefore, the endogenous α -ecdysone in each fraction was determined by radioimmunoassay and was observed to be the same for all fractions (concentrations $\sim 2 \times 10^{-2}$ of the ^3H - α -ecdysone used for the assay).

Schistocerca gregaria (H. Rees, personal communication) exhibited C-20 hydroxylase activity.

To characterise the C-20 hydroxylase as a mixed function oxidase, it was necessary to demonstrate that the hydroxylation was enzymatically mediated. Enzyme activity profiles under varying regimens of temperature and pH were obtained, with the observed optima being 30 °C and pH 7.5, respectively. The reaction was not affected by most monovalent and divalent cations, but Mg^{2+} ions inhibited hydroxylation (up to 25%) at concentrations as low as 10 mM. Time course analysis of substrate conversion revealed a linear response up to 60 min with a plateau obtained at 90 min. When increasing concentrations of mitochondria were incubated with fixed levels of substrate, proportionate increases in the hydroxylation of substrate to product were observed. Hyperbolic saturation kinetics were obtained when increasing levels of substrate were incubated with fixed concentrations of mitochondria. An apparent K_m of $1.6 \pm 0.7 \times 10^{-7}$ M (mean $\pm$ s.d.) was derived from Lineweaver-Burk plots obtained by linear regression analysis of saturation kinetics data (Fig. 2). Using an *in vivo* kinetics system, a similar K_m has been reported for the C-20 hydroxylase of the fly

*Calliphora stygia*¹³. Chemical identification of β -ecdysone as the product of this putative C-20 hydroxylase was attained by acetylation finger printing of labelled product³ and ultimately by mass spectrometry of 1.0 μ g unlabelled material (m/e 462, 444, 426, 408, 345 and 328).

Substrate specificity of the C-20 hydroxylase is at present being assessed using a variety of steroids and ecdysone analogues. Preliminary results suggest that there is a high degree of specificity which depends on the number and position of hydroxyl groups, and more importantly, on the stereochemistry of the AB ring and side chain.

To demonstrate that the mitochondrial α -ecdysone C-20 hydroxylase is a cytochrome P-450 mixed function oxidase, the following criteria were used: dependence on NADPH (using an exogenous NADPH or NADPH generated by a glucose-6-phosphate dehydrogenase system¹⁴ or NADPH generated by Krebs cycle intermediates¹⁵); inhibition by CO^{7,8}, metyrapone (Metopiron)¹⁶, aminogluthethimide (Elipten)¹⁶ and *p*-chloro-mercuribenzoate¹⁴; and a CO difference spectrum¹⁷. The C-20 hydroxylase activity was dependent on NADPH or NADPH generating systems as electron donors, with NADH being only about 25% as effective (Table 2). The C-20 oxygenation of α -ecdysone was efficiently inhibited by CO (Table 2), *p*-chloro-mercuribenzoate ($I_{50} = 5.0 \times 10^{-7}$ M), aminogluthethimide ($I_{50} = 1.0 \times 10^{-5}$ M) and metyrapone ($I_{50} = 5.0 \times 10^{-6}$ M). Metyrapone acts as a competitive inhibitor with an apparent K_i of 3.9×10^{-6} M (Fig. 2). The CO difference spectrum obtained for the CO-bound sodium dithionite-reduced mitochondria was similar to those obtained from vertebrates with an absorption maximum at A_{450} (Fig. 3).

Table 2 Effect of various co-factors and gases on the oxygenation of α -ecdysone by fat body mitochondrial C-20 hydroxylase

Co-factor	Relative C-20 hydroxylase activity
NADPH	1.0
NADP	0.07
NADH	0.27
NAD	0.01
ATP	0.05
ADP	0.03
NADPH-generating systems:	
Glucose-6-phosphate dehydrogenase	1.0*
Krebs cycle intermediates:	†
Malate	0.13
Malate + NADP	0.59
Fumarate + NADP	0.11
Isocitrate + NADP	0.12
Gases	
Air	1.0
Ar	0.97
CO ₂	0.05‡
CO	0.38
N ₂	0.95
O ₂	0.86

The method for assaying C-20 hydroxylase activity was as in Table 1. A dose response was determined for all cofactors at concentrations from 2.0×10^{-7} to 4.0×10^{-4} M. For NADPH and NADH, C-20 hydroxylase activity was dose dependent with a $V_{max/2}$ for NADPH of 6.0×10^{-6} M. Maximum oxygenation with NADH was not obtained at concentrations up to 4.0×10^{-4} M. For the rest of the cofactors, hydroxylase activity did not increase significantly above background. Gases were introduced to the standard assay system by bubbling at a flow rate of 35 ml min⁻¹ for 2 min. Following the introduction of gases and incubation, O₂ was present at a concentration of approximately 60 nmol ml⁻¹, which was 25% of the O₂ concentration of controls incubated in the absence of added gas. Stoichiometrically, this is a 5.4×10^3 excess of O₂ for the C-20 hydroxylation of α -ecdysone and precludes oxygen depletion as an explanation for the observed changes in C-20 hydroxylase activity in these gas studies.

*A glucose-6-phosphate dehydrogenase NADPH-generating system provided maximal C-20 hydroxylase activity at 4.0×10^{-6} M NADPH generated.

†Krebs cycle intermediates (2×10^{-3} M) were used to generate NADPH from exogenous NADP (4×10^{-4} M, equivalent to the NADPH in the hydroxylase assay). The ultimate effect of the Krebs cycle intermediates is apparently to provide an increased reduced cofactor (NADPH) pool for hydroxylase activity, as evidenced by the requirement for NADP with the Krebs cycle intermediates. Succinate, citrate, α -ketoglutarate and oxaloacetate in the presence of NADP did not increase hydroxylase activity above that observed for NADP alone.

‡Low conversion in the presence of CO₂ was probably due to acidification of the assay mixture by CO₂ to pH 6.2, which is below the minimum pH (6.5) for preservation of C-20 hydroxylase activity.

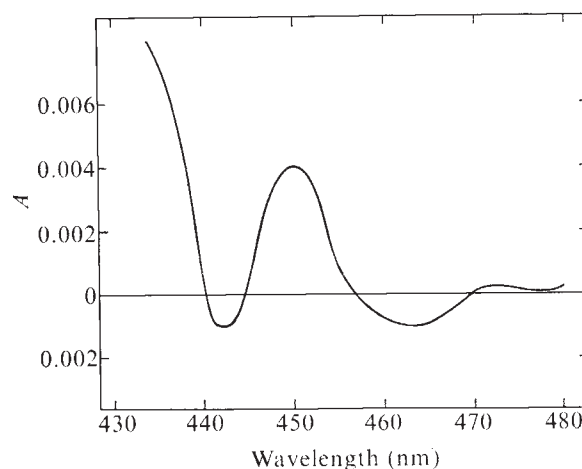


Fig. 3 A CO difference spectrum of sodium dithionite-reduced fat body mitochondria. The sample cell contained a suspension of dithionite-reduced mitochondria saturated with CO, whereas the reference cell contained only dithionite reduced mitochondria. The difference spectrum was obtained using a Cary 14 spectrophotometer equipped with a transmission scatter attachment.

The above studies demonstrate that the α -ecdysone C-20 hydroxylase is located in the insect fat body mitochondria and that cytochrome P-450 is probably the oxygen activator for the oxygenation of α - to β -ecdysone. This is the first report implicating a cytochrome P-450 mixed function oxidase in the oxygenation of steroids in insects, and in arthropods in general.

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Potential-dependent transition temperature of ionic channels induced by glutamate in locust muscle

RECENT work has emphasised the important role which the state of the membrane has in the function of its transport and enzyme systems^{1,2}. At various synapses the action of transmitter substances is accompanied by 'membrane noise' associated with the opening and closing of ionic channels. Analysis of this noise gives an estimate of the mean conductance and lifetime of the single open channel^{3–9}. In locust muscle we have found that the mean lifetime (τ) of channels, activated either by the natural transmitter or by externally-applied glutamate, is prolonged as the temperature is lowered, while the conductance (γ) of the single channel is decreased. The dependence of γ and τ on temperature shows a change in slope at a temperature which itself depends on the membrane potential. These results are possibly due to a potential-dependent phase transition in the membrane around the channel causing an abrupt change in the operation of the gating molecule and conductance-determining portion of the channel.

Distal fibres of the metathoracic extensor tibiae muscle¹⁰ of the locust (*Schistocerca gregaria*) were voltage-clamped using two intracellular microelectrodes. L-glutamate was applied ionophoretically from a micropipette to a junctional site^{7,11}. The bathing medium was as previously described and contained increased Mg^{2+} and decreased Ca^{2+} in experiments where the nerve was stimulated. Bath temperature was controlled with a Peltier cooling device and monitored with a thermistor placed near the muscle bundle under investigation. Bath reference electrodes were isolated through agar bridges to reduce temperature-induced junction potentials. Temperature readings were accurate to within $\pm 0.5^\circ\text{C}$.

The energy spectrum of the current noise obtained during steady application of glutamate to junctional receptors in locust muscle results from statistical fluctuations in the opening

of ionic channels, and agrees well with a first order process which follows the relationship⁷

$$S(f) = \frac{2\gamma\mu_g/\alpha}{1 + (2\pi f/\alpha)^2} \quad (1)$$

characterised by the independent parameters γ and α . Under a specific hypothesis for the operation of the glutamate-sensitive receptor complex⁷, similar to that suggested for acetylcholine receptors in vertebrate muscle^{3,4,12}, γ and α are respectively the conductance and closing rate constant of the single ionic channel. The mean life-time of the open state is given by $\tau = 1/\alpha$.

For a given mean conductance (μ_g) lowering the temperature prolongs the mean life-time (τ) of the open channel (seen as a shift in the cut-off frequency of the noise spectrum) and reduces the variance of the noise, by decreasing the conductance of the single channel (seen as a reduction in the zero frequency asymptote, $S(0)$, of the spectrum). Apart from altering γ and α , changing the temperature did not alter the spectral relationship (equation 1) over the range studied ($2\text{--}25^\circ\text{C}$).

Figure 1 shows the temperature-dependence of γ (the single-channel conductance), and α (the rate constant for channel closing) at a fixed membrane potential. γ and α decrease exponentially with the inverse of the absolute temperature ($^\circ\text{K}$) at all temperatures studied but, at about 6°C the slope of the

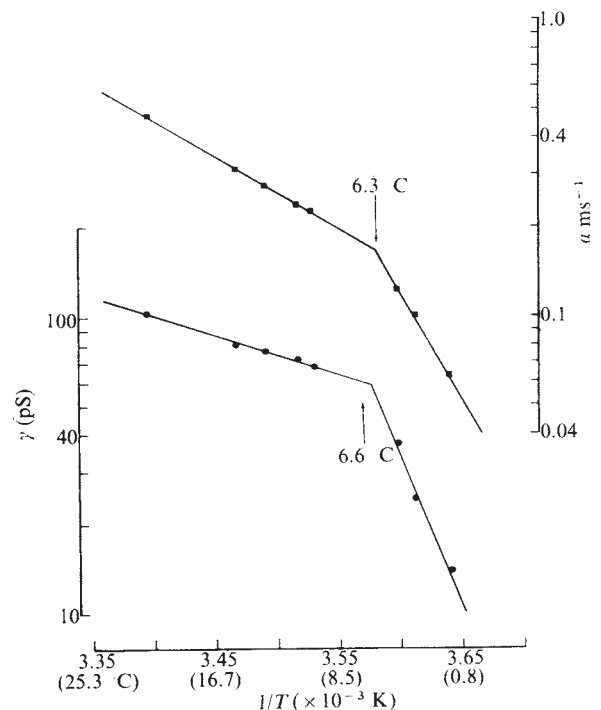


Fig. 1 Temperature dependence of the glutamate-induced single-channel characteristics. The channel closing rate constant (α (ms^{-1})) and the single channel conductance (γ (pS)) are plotted semilogarithmically against T^{-1} (Kelvin). Temperatures in $^\circ\text{C}$ are given in parentheses. Values were obtained during steady ionophoretic application of glutamate by fitting the membrane current fluctuation spectra to the relationship in equation (1). Background noise spectra were subtracted before fitting. Transition temperature for α is 6.3°C and for γ is 6.6°C , for a membrane potential of -100 mV . The agreement between the data and the solid lines suggests that α and γ behave according to a reaction scheme in which the apparent activation energy increases sharply below the transition temperature. Data is from six voltage-clamped fibres. Each point represents the spectral parameters of the average spectra from 20 noise segments each digitalised at 2,048 Hz in 1,024 point segments over 0.5 s. Spectral estimates were calculated using an F.F.T. algorithm.

relationship changes abruptly. The values for γ and α may be fitted to the relation depicted by the solid lines in Fig. 1, which represent the behaviour expected of first-order rate processes. For α , this hypothesis is governed by the relationship^{1,2}

$$\alpha = \hat{\alpha} e^{-E_a/RT} \quad (2)$$

where E_a is the energy required for movement of the gating molecule where $\hat{\alpha}$ is constant, T is absolute temperature in °K and R is the gas constant. The lines drawn for α in Fig. 1 show the relationship for $T > 6^\circ\text{C}$, where $\alpha_1 = 1.15 \times 10^8 \text{ ms}^{-1}$, $E_{a1} = 11.4 \text{ kcal mol}^{-1}$ and for $T < 6^\circ\text{C}$, $\alpha_2 = 1.37 \times 10^{25} \text{ ms}^{-1}$, $E_{a2} = 33.3 \text{ kcal mol}^{-1}$.

If the single-channel conductance is envisaged as being determined by the rate of transfer of ions, v , across an energy barrier, E_v , in the postsynaptic membrane, then the resulting first order rate process may be represented by

$$v = \hat{v} e^{E_v/RT} \quad (3)$$

which yields the unitary conductance

$$\gamma = vZF/(V - V_{eq})A \quad (4)$$

where Z is valence, F is Faraday's constant and A is Avogadro's number. The lines drawn for γ in Fig. 1 show the relationship for $T > 6^\circ\text{C}$ where $E_{\gamma 1} = 6.12 \text{ kcal mol}^{-1}$ and for $T < 6^\circ\text{C}$, $E_{\gamma 2} = 46.5 \text{ kcal mol}^{-1}$. The transition temperature for α is 6.3°C (at $V_m = -100 \text{ mV}$) and for γ is 6.6°C ($V_m = -100 \text{ mV}$). Transition temperature values for α and γ are practically the same within the accuracy of our measurements.

At acetylcholine receptors in frog muscle⁴ and at glutamate receptors in locust muscle⁷, the postsynaptic excitatory junctional current (e.j.c.) evoked by nerve stimulation has an exponential decay with a time constant ($\tau_{e.j.c.}$) identical to that which characterises the $1/f^2$ component of the current noise spectrum; $\tau_{e.j.c.} = 1/2\pi f_c = \alpha/2\pi$, where f_c is the half-power frequency of the spectrum. This allowed us to investigate the temperature dependence of α for channels activated by the natural transmitter. This technique was also used to study more fully the behaviour of the rate constant close to the point of transition, and particularly its dependence on membrane potential.

Figure 2 shows a semilogarithmic plot of $\tau_{e.j.c.}^{-1}$ as a function of T^{-1} at two membrane potentials. The relation follows two straight lines at each potential without obvious curvature near the critical temperature. This may suggest a discrete change in a single rate limiting energy barrier rather than a dual process with different temperature dependencies, or a single kinetic scheme in which the activation energy is a continuous function of temperature. The results fit well the relationship $\tau = \tau_e^{(E_a + \eta(T))/RT}$, which is predicted from an energy barrier model for the closing of channel gates (unpublished) in which the free energy change of the system is coupled to the electric field through a temperature-dependent dipole (η). Values for the apparent activation energies and transition temperatures are given in Table 1. As is seen in Fig. 2, the transition temperature depends on membrane potential in such a way that

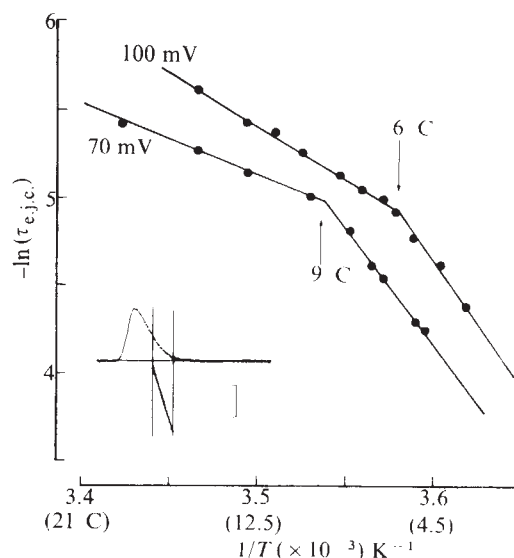


Fig. 4 Arrhenius plot of temperature dependence of the time constant of decay of nerve-evoked e.j.c.s in two muscle fibres clamped at -100 mV and -70 mV respectively. As shown in the inset, digitalised e.j.c.s were plotted semilogarithmically and a least squares line fitted to the decay phase gave $\tau_{e.j.c.}$ (calibration bar 80 nA ; e.j.c. is 70 ms full sweep; V_m , 100 mV ; T , 20°C). Each point on the graph represents at least 50 such determinations. In all cases the standard error of the mean is smaller than the symbol. Transition temperatures obtained from the intercepts 6.1°C at $V_m = 100 \text{ mV}$ and 9.1°C at $V_m = 70 \text{ mV}$. Fibre input resistances were $2.1 \text{ M}\Omega$ (V_m , 100 mV) and $5.2 \text{ M}\Omega$ (V_m , 70 mV). Clamp efficiency was better than 99.6% at 0 Hz and better than 90% at 1.6 kHz . Temperatures ($^\circ\text{C}$) are given in parentheses.

depolarisation causes a shift in transition temperature to higher values. Transition temperature seemed to show a linear dependence on membrane potential.

We conclude that the apparent activation energies of the rate processes characteristic for the synaptic gate and the conductance-determining portion of the ion channel undergo a discrete change in value at a transition temperature which depends on membrane potential.

One interpretation of the break in the Arrhenius plots shown in Figs 1 and 2, is that it reflects a conformational change in the receptor—and associated ionic channel—similar to that observed in some proteins¹³. Another possibility is that the break is a consequence of a lipid phase transition in the membrane^{1,2}. A change in the state of membrane lipids has already been invoked to explain the effects of alcohols on miniature endplate currents in toad muscle¹⁴. But, the possibility that the fluidity of the lipids associated with the receptors may influence their function, should be considered only as a speculation until direct measurements are made on the nature and state of the lipids surrounding the receptors.

If the break in the temperature dependence of α and γ is due to a lipid phase transition, then several questions arise. For

Table 1 Apparent activation energies (E_{a1} : above transition temperatures; E_{a2} : below transition temperatures) for the rate constant for channel closing α and for the rate constant for the transmembrane flow of ions v

	Rate constant	V_m (mV)	E_{a1} (kcal mol ⁻¹)	Q_{10}	E_{a2} (kcal mol ⁻¹)	Q_{10}	E_{a1}/E_{a2}	Transition temperature ($^\circ\text{C}$)
Glutamate	α	-100	11.4	1.8	33.3	6.3	1/2.9	6.3
	v	-100	6.1	1.2	46.5	7.2	1/7.6	6.6
Transmitter	α	-70	8.2	1.7	25.9	5.4	1/3.2	9.1
	α	-100	11.9	2.2	28.1	6.6	1/2.4	6.1

Values are averages based on fitting the relationships $\alpha = \hat{\alpha} e^{E_a/RT}$ and $v = \hat{v} e^{E_v/RT}$ to the data for $\alpha(V_m, T)$ and $\gamma(T)$ respectively. Values obtained in response to glutamate or in response to neurally released transmitter as indicated.

instance, in cell membranes the transition usually occurs over a broad change in temperature and the Arrhenius plot may show two breaks and three segments: one corresponding to the transition range, and one above and one below the transition. Our plots seem to have only two segments. This could indicate that the transition occurs over a narrow range in temperature, perhaps as a result of the close packing and relative immobility of the receptors which may be anchored in the synaptic membrane. Alternatively, the lower end of the transition may not have been reached.

The similarity of the transition temperatures for single channel conductance (γ) and for the rate constant of channel closing (α) may indicate that both processes are affected by the same mechanism. Perhaps the glutamate recognition site of the receptor, and that part of the receptor which contains the main energy barrier for ion translocation, lie close together and penetrate well into the membrane so that both portions may be altered by changes in lipid structure. In embryonic chick muscle⁹ and at mammalian endplates¹⁵, a break has been observed in the relationship between temperature and channel conductance, but not in that between temperature and lifetime of the channels induced by acetylcholine. The reason for this apparently different behaviour of glutamate and acetylcholine receptors is not yet clear. Perhaps the recognition site of the acetylcholine receptor lies more in the aqueous external face of the membrane, and relatively distant from the membrane-embedded portion which controls the ionic flux; in which case a lipid phase transition may not exert an important influence on the drug-receptor kinetics.

If the transition we described results from a phase change in the lipid associated with the receptor, then the voltage dependence of the transition could result from a coupling of the membrane electric field to the charge distribution in the lipid component.

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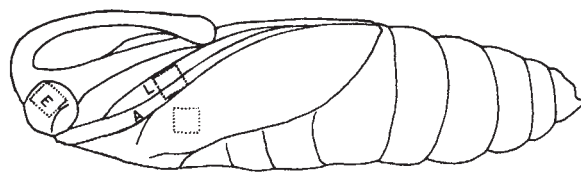


Fig. 1 The tissue regions used for grafting in the study of inter-tissue affinities. Dorsal is to the bottom. Four different epidermal tissues were selected: wing (W), eye (E), antenna (A), and mesothoracic leg (L). The graft sizes varied slightly but were usually 1.5×1.5 mm, each consisting of approximately 6,000 cells. All grafts were removed from recently pupated animals (6–15 h after pupation); at this stage the cells showed no evident morphological differences in the light microscope. Because of the proximity of antenna and leg epidermis to each other, these two epithelia were removed and transferred as a single graft unit. In all transpositions (except between leg and antenna) the original orientations of the grafts were retained. In the contralateral exchanges between antenna and leg, which were necessitated by the fact that the two tissues were handled only in combination, the dorsoventral axes of these tissues were reversed without concomitant reversal of their proximo-distal axes. Therefore, the possibility existed that misalignment of the dorsoventral axes of host and graft influenced their mutual affinities. But, in control experiments involving similar contralateral exchanges between either an antennal pair or a leg pair, differences in epidermal properties were never manifested.

for this sorting out of cells in terms of differences in their adhesiveness. Although arranged in monolayers, insect epidermal cells seem to behave similarly when their normal spatial organisation is disrupted. The regrouping of disaggregated *Drosophila* imaginal disk cells according to epidermal type and the failure of grafts from one epidermis to form stable contacts with cells of another epidermal tissue have both been attributed to sorting out of unlike cells^{4,5}. Yet some insect epidermal cell types fail to segregate completely from one another; even when disaggregated and mixed, cells from genetically marked leg and antenna disks are known to form some integrated patterns⁶. It is possible that different degrees of adhesiveness exist for various combinations of two different epidermal types, the adhesive properties not being uniquely specified for each tissue. With this possibility in mind, exchanges between grafts and hosts of various pupal epithelia of the moth, *Manduca sexta*, were performed. The affinity relationships observed reflected the existence of covert cell surface differences in these epidermal populations whose component cells at the time of grafting are determined but undifferentiated. The findings suggest that the cell surface properties are not tissue type specific but are shared to various degrees by the different epidermal tissues.

Animals for experiments were reared, selected, and treated as described in Nardi and Kafatos⁷. The pupal tissue regions chosen for the study are shown in Fig. 1. Contact between two different tissues was scored in animals just before the time of adult emergence from the pupal cuticle (3 weeks after pupation) and varied from contact involving little or no decrease in original graft perimeter to the formation of completely free, differentiated graft vesicles. By carefully removing the overlying pupal cuticle from the grafting sites, all graft tissues were recovered; otherwise unattached vesicles would have been lost during adult emergence. Only the presence or absence of contact between host and graft was scored, and the results were expressed as fractions of grafts that maintained cell-cell contact with host tissues. Because of the ease in distinguishing free vesicles from attached graft tissues under a dissecting microscope, ambiguities in scoring were avoided. Figure 2 shows typical grafts that were scored as having maintained cell contact with the host.

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Are cell affinity relationships for insect epidermal tissues evidence for combinatorial control?

The segregation of determined, yet undifferentiated, cells in aggregates consisting of cells from various vertebrate embryonic structures represents a high degree of specificity in cellular recognition^{1–3}. Steinberg's differential adhesiveness model² has been particularly successful in accounting

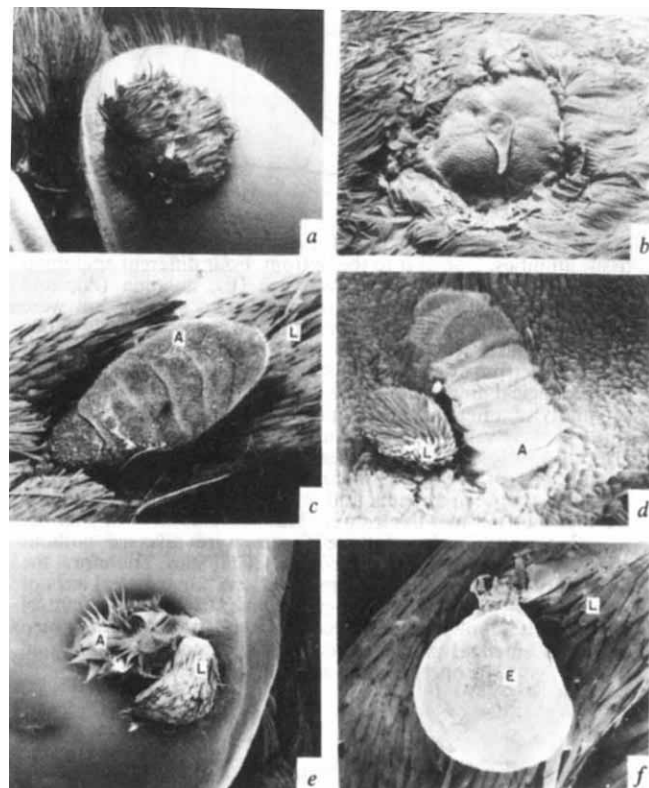


Fig. 2 Scanning electron microscope photographs of representative grafting results. *a*, Extensive contact exists between the wing cells and the eye epidermis ($\times 28.5$). *b*, Eye tissue transposed to the wing ($\times 42$). *c*, Female antenna (A) tissue adhering to the leg ($\times 63$). *d*, Leg (L) tissue and male antenna epidermis (A) tenuously attached to the wing surface ($\times 45$). *e*, Male antenna epidermis and leg epidermis grafted to eye. Only the antenna tissue is attached to eye; the leg epidermis is attached to the antenna ($\times 39.5$). *f*, An inverted eye vesicle attached to the leg ($\times 120$).

No evidence of vesicle formation was found for grafts exchanged within a given epithelium (Table 1); this was true whether grafts were transferred between different positions in the same epidermis or exchanged between corresponding sites of two pupae. Moreover, a particular

epithelial tissue shows no consistent tendency either to fail or succeed in establishing contact with different epithelial cell populations. These facts argue against the possibility that the observations reflect grafting artefacts rather than cell surface differences.

Several notable features emerge from Table 1. First, there is considerable disparity in affinities. For example, values range between the extreme values of 1.0 for antennal grafts transposed to leg epidermis and 0.0 for leg epidermis transposed to eye tissue ($P < 0.01$). A given graft does not show the same affinity for all the host sites examined. This is clear for exchanges of $L \rightarrow E$ and $L \rightarrow W$, these values being 0.0 and 0.6, respectively ($P < 0.01$). Second, in a reciprocal exchange the affinity value is a function of which tissue is the graft and which is the host. For most of the pairs listed, the two affinity measures are not equivalent (with the exception of the eye, leg exchanges). This disparity is well illustrated by a comparison of the 0.4 affinity value for $L \rightarrow A$ to the value of 1.0 for $A \rightarrow L$ ($P < 0.01$). Other reciprocal exchanges show statistically significant differences in affinity values. Third, evidently no correlation exists between affinity and spatial arrangement of *Manduca* tissues. The distance separating the eye from the leg is no greater than that separating the eye from the wing region; yet the fractional values for the $E \rightarrow L$ and $E \rightarrow W$ exchanges differ by almost a factor of ten.

The affinity asymmetry for two given tissues is most readily accounted for by a model which deals with pairs of tissues that are either at configurational equilibrium or that are approaching it through minimisation of their surface free energies^{2,7}. In applying the model to an exchange between A and B tissues whose cells are arranged in monolayers, B graft cells, after a given time period, will show more extensive contact with A host cells than that displayed by A grafts with B host tissue provided that certain A-B relationships hold true, that is, the cohesiveness of A cells exceeds the cohesiveness of

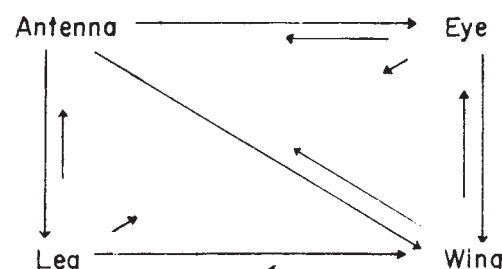


Fig. 3 Mutual affinities of various *Manduca* epidermal tissues. The unidirectional arrows between two epithelia point from the graft to the host tissue; and the relative magnitudes of the arrows for a given exchange reflect relative affinities for that exchange.

Table 1 Fraction of grafts which form stable contacts with host tissues

Graft	Host	No. of exchanges	No. of adhering grafts	Fraction of grafts adhering (affinity value)	Significance level of differences in affinity values for a given exchange between two epidermal types
W	W	34	34	1.0	—
L	L	34	34	1.0	—
A	A	34	34	1.0	—
E	E	34	34	1.0	—
W	L	35	11	0.3	$P < 0.03$
L	W	35	21	0.6	
W	A	35	14	0.4	$P < 0.01$
A	W	35	32	0.9	
W	E	35	8	0.2	$P < 0.02$
E	W	35	19	0.5	
L	A	46	17	0.4	$P < 0.01$
A	L	46	46	1.0	
E	L	33	1	0.0	$P > 0.10$
L	E	33	1	0.0	
E	A	33	7	0.2	$P < 0.01$
A	E	33	23	0.7	

B cells. This situation is analogous to the asymmetrical, albeit three-dimensional, interaction of two immiscible liquids A and B, one of which exists either as a liquid drop or a film on the surface of a liquid substrate. A drop of A will form as long as the sum of the surface energy for A and the surface energy of the A-B interface exceeds that for substrate B; but if the surface energy of the substrate A exceeds the total surface energy for drop B and the A-B interface, then B augments its contact with the substrate. In speaking of surface energy for monolayers of cells, we are dealing with the energy for those lateral cell surfaces which establish contact with adjacent cells. This energy is a reflection of the cells' cohesiveness.

According to the combinatorial model of Kauffman which deals with the phenomena of determination and transdetermination in *Drosophila*, the global directionality that is manifested by the transdetermination pathway and the

affinity relationships in Fig. 3 (that is, the prevailing orientation for every exchange is towards wing) may indicate the existence of underlying bistable control systems⁸. Another model involving binary developmental decisions—the selector gene model—is based on genetic analysis of *Drosophila* development and was independently developed by Garcia-Bellido⁹. In this combinatorial model, selector genes comprise that set of genes controlling developmental pathways and determination. Of major relevance to the study discussed in this report is that work with one such gene, the wild-type allele of the *engrailed* locus, has suggested that the activity of selector genes supplies cells with distinctive surface labels that govern cellular recognition¹⁰.

Although the nature of the relationship between determination and the mutual adhesiveness of different epidermal cells remains obscure, the striking correspondence between these two phenomena suggests that, regardless of the location of the determinative factors, the cell surface properties are probably closely associated with decisions involving cell determination.

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Control of induction of tubulin synthesis in *Chlamydomonas reinhardtii*

REMOVAL of the flagella from the biflagellate alga *Chlamydomonas reinhardtii* results in the rapid production of new flagella^{1–3}. Flagellar regeneration is 80–85% complete within 60 min (Fig. 1). Indirect evidence obtained from *in vitro* translation of polyribosomes and mRNA from deflagellated gametes¹ has indicated that flagellar regeneration is accompanied by a strong but transient induction of tubulin synthesis. Kinetic analysis showed that tubulin mRNA could be detected in polyribosomes in as little as 15 min after deflagellation. Since the cytoplasm contains a reserve of tubulin of sufficient size to supply at least one-third to one-half of the tubulin subunits required for flagellar reassembly^{1,3}, the rapid initiation of tubulin synthesis following deflagellation suggested the existence of a highly sensitive system for detecting and responding to the cell's anticipated need for additional tubulin. As an approach to seeking such a mechanism, we have carried out studies to determine if the depletion of tubulin reserves during flagellar outgrowth might provide the signal for the induction of tubulin synthesis. We present here two types of experimental evidence which clearly demonstrate that the induction of tubulin synthesis is independent of the utilisation of tubulin reserves during flagellar regeneration.

Comparison of the spectrum of proteins produced by control and deflagellated gametes during a 30 min pulse label with ³⁵S-H₂SO₄ shows that deflagellation results in a marked

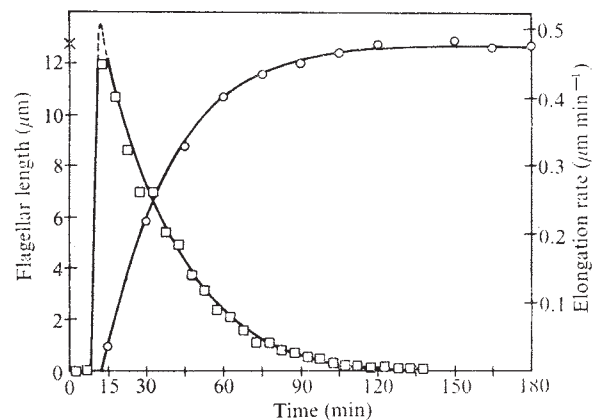


Fig. 1 Flagellar regeneration in gametes of *C. reinhardtii*. Gametic cells were deflagellated by vigorously stirring cells at pH 4.7 for 1 min and then raising the pH of the medium to 7.0 (ref. 3). At various times after deflagellation, cells were fixed with 1% glutaraldehyde and 10 mM potassium phosphate (pH 6.8), and flagellar length measured as described previously¹. $\times$, Initial flagellar length; $\circ$, flagellar length; $\square$, rate of flagellar elongation. For preparation of gametic cultures, synchronously grown vegetative cells of strain 137c near saturation density were transferred to a nitrogen deficient media, either medium 2-N (ref. 1) or medium 2-N without sulphate (designated medium V) in which an equivalent level of MgCl₂ was substituted for MgSO₄. If gametes were formed by overnight incubation in medium 2-N, they were rinsed and re-suspended in medium V 1 h before deflagellation (or before labelling with ³⁵S-H₂SO₄).

induction of the α (56,000 molecular weight) and β (53,000 molecular weight) subunits of tubulin (see tracks 2 and 6, Fig. 2). Chloramphenicol is added during *in vivo* labelling to prevent the synthesis of the 55,000 molecular weight subunit of ribulose biphosphate carboxylase (tracks 3 and 4) that would otherwise interfere with the analysis of tubulin synthesis (tracks 1 and 5 compared with 2 and 6). The magnitude of tubulin induction following deflagellation is indicated by densitometric analysis of the radioautographs presented in Fig. 3 (tracks 1–4). In the time intervals 15–45 min, 45–75 min, 105–135 min and 165–195 min, tubulin synthesis accounts for 18.1, 14.5, 7.7 and 3.2 of total cellular protein synthesis (4% has been subtracted from each value to compensate for 'background' incorporation in the 53–56,000 molecular weight region of gels containing proteins synthesised by non-deflagellated cells).

The above *in vivo* labelling experiments provide a direct demonstration of the rapid and extensive nature of tubulin induction following flagellar excision. Additional experiments (not presented) have shown that the initiation of tubulin synthesis occurs between 12 and 15 min after deflagellation. Thus, if *de novo* synthesis of tubulin mRNA is required for tubulin induction, as suggested by the observation that tubulin mRNA is not detectable in non-deflagellated cells¹, then the time requirements for mRNA transcription, processing and transport¹⁰, dictate that the signals required for the activation of tubulin genes must be generated by reactions intimately associated with the deflagellation event. Since flagellar regeneration is not observed until 10–12 min after deflagellation (Fig. 1), the kinetic data suggest that utilisation of tubulin subunits for flagellar outgrowth cannot serve as a feedback signal for the induction of tubulin synthesis. As a further, more direct, test of this question, we have treated cells with a variety of compounds that block flagellar regeneration to determine if these treatments have any effect on tubulin induction. Results presented in Fig. 3 show that treatment of cells with 1 mM isobutylmethylxanthine (IBMX) either 15 min before deflagellation (tracks 5–8) or immediately after deflagellation (tracks 9–12) has no effect on the early phases of tubulin induction even though IBMX, like other methylxanthines^{11,12}, completely blocks flagellar outgrowth. Treatment of deflagellated cells

with colchicine, chloro-isopropyl-*n*-phenylcarbamate, 50 mM potassium phosphate (pH 6.8) and other agents that prevent flagellar regeneration, provide nearly identical results to those obtained with IBMX (not shown). These findings provide clear evidence that utilisation of tubulin reserves for flagellar outgrowth cannot serve as a signal for the initiation of tubulin synthesis.

Finally, it may be noted that although intracellular tubulin concentrations do not seem to control the early phases of tubulin synthesis, indirect evidence indicates that increasing reserve levels may govern tubulin production during the later stages of flagellar regeneration. A comparison of data in Figs 1 and 3 shows that relatively high levels of tubulin production continue well beyond the time when flagellar outgrowth is essentially complete and therefore suggests that tubulin reserves accumulate during the late stages of regeneration.



Fig. 2 Autoradiographs of SDS-polyacrylamide gels containing *in vivo* labelled proteins from deflagellated *Chlamydomonas* gametes. Cells were labelled with $^{35}\text{S}\text{-H}_2\text{SO}_4$ using modifications of procedures developed by Lefebvre and Rosenbaum (personal communication) and other laboratories⁵⁻⁸. Approximately 10^7 sulphate-starved cells in 0.5 ml were labelled for 30 min in 100 μCi carrier-free $^{35}\text{S}\text{-H}_2\text{SO}_4$. Non-deflagellated cells were incubated without protein synthesis inhibitors (track 1); with 100 $\mu\text{g ml}^{-1}$ chloramphenicol to inhibit chloroplastic protein synthesis (track 2); with 10 $\mu\text{g ml}^{-1}$ cycloheximide (track 3) or with 10^{-4} M 2-(4-methyl-2,6-dinitroanilino)-*N*-methylpropionamide (ref. 9, MDMP) (track 4) to inhibit protein synthesis by 80S cytoplasmic ribosomes. Cells deflagellated by the pH shock method were incubated for 15-45 min after deflagellation in the absence (track 5) or presence of chloramphenicol (track 6). After incubation, a 5- μl aliquot of each sample was taken for hot TCA determination of ^{35}S incorporation into protein¹. The remainder of each sample was precipitated with 5 ml acetone. For analysis on SDS-polyacrylamide gels, the acetone powder from each sample (which can be stored indefinitely) was dissolved in 2% SDS, 5% mercaptoethanol, 10% glycerol, 60 mM tris (pH 7.6), and an aliquot with 2×10^6 c.p.m. (except for tracks 3 and 4) electrophoresed on 9% SDS-polyacrylamide gels as described previously¹. Autoradiographic exposure of the dried gel was for 12 h.

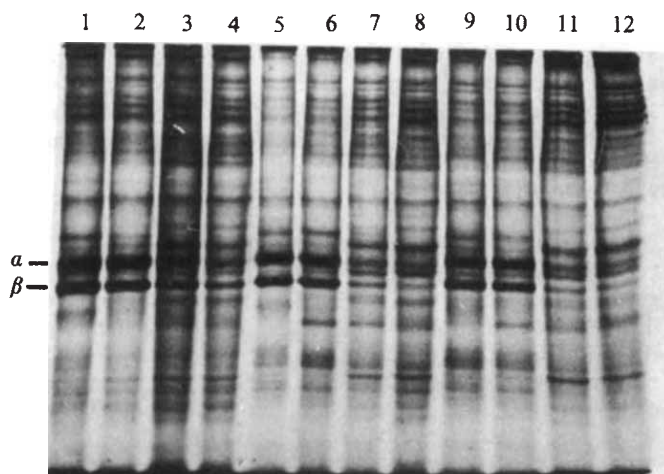


Fig. 3 Tubulin synthesis following deflagellation of control or IBMX-treated cells. Deflagellated cells were labelled for 30-min intervals at 15-45, 45-75, 105-135 and 165-195 min after deflagellation in the absence (tracks 1-4) and presence of 10^{-3} M IBMX given either 15 min before deflagellation (tracks 5-8) or immediately after deflagellation (tracks 9-12). Labelling with $^{35}\text{S}\text{-H}_2\text{SO}_4$ in the presence of chloramphenicol was as described in Fig. 2.

Thus, the earlier than normal shut off of tubulin synthesis in IBMX-treated cells (Fig. 3, tracks 5-12 compared with 1-4) suggests that a feedback mechanism responsive to increasing tubulin concentrations, could be responsible for the ultimate repression of tubulin synthesis during flagellar regeneration.

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Erratum

In 'Stability of Lotka-Volterra systems' (*Nature*, **264**, 381; 1976) the name of the co-author, J. S. Semura, was omitted.

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matters arising

Geomagnetism and climate

FAIRBRIDGE¹ correlates the Gothenburg geomagnetic excursion (about 13,500 yr BP) with climatic changes associated with the North American glacial record, eustatic changes in sea level, and changes in rainfall and river discharge in the Nile Valley. Such a correlation is interesting considering the possible resulting climatic effects as suggested by Harrison and Prospero². But, Fairbridge in presenting his case ignores the cyclical nature of the continental and oceanic climatic histories. The Holocene, viewed as a whole, is characterised by alternating intervals of glacial advance and retreats. When viewed in this way the correlation of the Gothenburg geomagnetic excursion with a small segment of the climatic history of the earth is hardly justified. Two examples illustrate the danger involved in this kind of correlation. Denton and Karlen³, from an examination of ¹⁴C dates on glacial termini, show that the dates of glacial maxima peak at 200–300, 2,800 and 5,300 yr BP which suggests a recurrence of major glaciers every 2,500 yr. Extrapolating backwards in time, this pattern suggests glacial advances peaking at about 7,800, 10,300, 12,800 and 15,300 yr BP. The Gothenburg excursion corresponds to the time of the Port Huron advance (12,700 to 13,000 yr BP), and/or the Cary/Port Huron interstadial (about 13,300 to 14,000 yr BP), and is no way a unique event when the whole record is viewed. The single correlation of geomagnetic and climatic events at 13,500 yr BP fails to explain similar climatic changes occurring with some regular frequency over the whole Holocene.

In the same way, Fairbridge cites the oxygen isotope record of the Greenland Ice Core in support of his hypothesis. He presents, however, only a small time interval of this record. The isotope record shows a long term climatic trend from 14,000 to 10,000 BP superimposed on which are major oscillations (ref. 4). Given the nature of this climatic record, the correlation of geomagnetic changes and climate at 13,500 yr BP seems purely coincidental.

Finally, it may be suggested that the oceans might provide a reasonable source of material from which a correlation of geomagnetic and climatic changes may be recognised. The oxygen isotope record of foraminifera from deep-sea cores in the Gulf of Mexico, for example, record a major influx of freshwater between 15,000 and

12,000 yr BP⁵. This resulted from the deglaciation of the Laurentide Ice Sheet and subsequent drainage through the Mississippi River Valley. Its correlation in time with the Gothenburg geomagnetic excursion seems pure chance when one examines the ¹⁸O record of deep-sea cores throughout the ocean. From such data⁶ one is unable to find any major climatic event over the interval of time in question (11–14,000 yr BP).

Thus while the correlation between geomagnetism and climate is interesting, there is no evidence to support it. The main difficulty with the data given by Fairbridge is that it fails to recognise the cyclical nature of the Holocene Climatic record. While many forcing functions may drive it, the case for geomagnetism as a driving mechanism is yet to be made.

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FAIRBRIDGE REPLIES—Hecht insists that I have ignored cycles in climatic history, but this is not so. Three major systems are identifiable: (1) the 200–250-Myr cycle (possibly galactic); (2) the 20,000–100,000-yr Milankovitch cycles (planetary orbits); and (3) the 11-yr (plus multiples) solar radiation cycle. Each is exogenetic. In contrast, the terrestrial response is greatly dependent on variable palaeogeography—an endogenetic control.

Hecht refers to the excellent glacial chronology of Denton and Karlen. I have made use of it in a paper entitled: 'An 8,000-yr palaeoclimatic record of the 'Double Hale' 45-yr Solar Cycle'¹. The major coolings on the other hand fall closer to 850-yr intervals, but I do not yet have a convincing casual correlation to offer. The variability does not at present justify Hecht's extrapolations.

In my Fig. 1, covering only the 'small interval' of 8,000 yr, I did not suppress evidence of other oscillations, but merely tried to stress the coincidence of the Gothenburg Event with the largest of them.

There still remains a 'chicken-and-egg' problem. The timing of the Gothenburg reversal (closer to 12,500 BP, according to Mörner, personal communications) puts it half-way through the last deglacial phase and thus suggests it was triggered by the massive asymmetric glacial unloading. The latter was catastrophically rapid in geological terms. Thus a general exogenetic global warming trend might trigger an endogenetic geomagnetic phenomenon that in turn introduced a brief cooling. Hecht is right to look at oceanic records, but not for oscillations of only a few centuries. For this information we need carefully analysed cores from lakes where rapid sediment accumulation is taking place.

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Palaeomagnetic stratigraphy of the Koobi Fora Formation, east of Lake Turkana (Lake Rudolf), Kenya

IN their recent article on the magnetostatigraphy of the Koobi Fora Formation of northern Kenya, Hillhouse *et al.*¹ noted that amendments to published correlations of the Koobi Fora stratigraphy were desirable but were unable to take this into account in the evaluation of their results. They noted that alternative dates were now available for the KBS Tuff but did not pursue the inference that if one published ⁴⁰Ar/³⁹Ar date was questionable, others might be similarly suspect. Some of their statements concerning the geographic location of tuffaceous outcrops were misleading.

An important offshoot from detailed investigation of the evolution of African Plio-Pleistocene Suidae has been the acquisition of correlative information of value both within the Koobi Fora region and between this and other African sites of similar age (J. M. Harris and T. D. White, in preparation, ref. 7). Suid correlative evidence suggests, for example, that the 'Tulu Bor' Tuff of Koobi Fora Area 102 (102/T.IV) is in disagreement with Cerling *et al.*², clearly younger than the type Tulu Bor Tuff of Area 129

(129/T.II) or undoubted correlative (116/T.I, 117/T.III) and is probably of similar age to the type KBS Tuff (105/T.II). A second significant fact to emerge is that the suid fauna from immediately subjacent or superjacent to the Tulu Bor Tuff is evolutionarily equivalent to that from the upper part of Shungura Member B at Omo. A third, equally important, consideration is the apparent absence from the Koobi Fora region of fossiliferous sediments yielding faunas equivalent to those from Shungura Members C-E. It is important also to remember that very few fossiliferous sequences from the Koobi Fora region can be shown to represent other than short time spans and that former lithologic correlations between geographically widely separated areas have to be treated cautiously.

It seems reasonably clear from available vertebrate evidence that the Tulu Bor Tuff is not, in terms of conventional radiometric dates, as old as 3.18 Myr (the date obtained for 116/T.I; ref. 3) and that the normal palaeomagnetic interval observed in that part of the sequence incorporating the Tulu Bor Tuff is probably that separating the Kaena and Mammoth events. Faunal evidence suggests interpretation 2 of Hillhouse *et al.* (Fig. 5 of ref. 1) is strongly to be preferred over interpretation 1. There is no need to postulate secondary magnetisation for the early part of the Area 102 succession (Fig. 4, ref. 1)—the observed polarity being consistent with faunal evidence that this part of the succession is equivalent in age to the normal sequence found immediately below and above the KBS Tuff. The suid faunas from the upper part of the Lower Member of the Koobi Fora Formation are evolutionarily equivalent to those from Omo Shungura Member G and Olduvai Bed I. The conventional K/Ar determination of Curtis *et al.*⁴ for the KBS Tuff and other tuffs from the upper part of the Lower Member agree with widely accepted conventional K/Ar dates for Shungura Member G and Olduvai Bed I. It is possible that ⁴⁰Ar/³⁹Ar step heating methods and fission-track determinations that provide an age of 2.4 Myr for the KBS Tuff (and correlates)^{5,6} are better chronometric measures—in which case suid evidence suggests that accepted ages for Shungura Member and Olduvai Bed I are too young by about half a million years. These alternative hypotheses require further research.

References by Hillhouse *et al.*¹ to the 'KBS Tuff' of Area 10 and magnetostratigraphic sections from the Tulu Bor Tuff to the 'KBS Tuff' in Area 131 need correction. Area 10 boundaries were redefined in 1974 and the 'KBS Tuff' referred to by Curtis *et al.*⁴ is to be found in Area 12 (12/T.II). The Tulu Bor does not crop out in Area 131. It is present in the closely adjacent Area 129 but here,

as at other Koobi Fora localities, faunal evidence indicates an appreciable hiatus (equivalent to Shungura Members C-E) between the Tulu Bor and superjacent (KBS = 129/T.IV) tuff. Moreover, to place data from Area 15 and Area 129 in the same magnetostratigraphic column (Fig. 4, ref. 1) implies firm lithostratigraphic correlation between two areas separated by several kilometres of dense bush cover. It implies also a measure of confidence in this correlation that is not shared by all the participants in the Koobi Fora Research Project.

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HILLHOUSE REPLIES—I thank J. M. Harris for reporting some important new developments concerning the fossil Suidae of the Koobi Fora Formation. These previously unpublished results suggest, as he points out, that a more refined interpretation of the magnetostratigraphy is now possible. As outlined by Harris, the faunal evidence apparently supports interpretation 2 of the magnetostratigraphy presented by Hillhouse *et al.*¹ (Fig. 5), but amendments to interpretation 2 are called for. For example, the normal polarity zone that includes the Tulu Bor Tuff can be correlated with the normal interval (2.92–2.99 Myr) separating the Mammoth and Kaena events, and should not be assigned to the end of the Gauss normal epoch (2.41–2.84 Myr) as in interpretation 2. Also, the absence from the Koobi Fora region of fossil faunas equivalent to those from Shungura Members C-E² suggests that sediments representing the upper half of the Gauss normal epoch and the lower part of the Matuyama reversed epoch are not present in the Koobi Fora Formation. The adoption of this amended interpretation depends on the strength of the evidence from the fossil Suidae weighed against radiometric dating and palaeontological data from a diverse selection of fauna. We eagerly await publication of Harris's results so the matter can be openly evaluated by geologists and palaeontologists.

Harris is correct in pointing out that because of the 1974 redefinition of area boundaries the 'KBS Tuff' referred to by Curtis *et al.*³ and by us is now in area 12. As Harris points out, the Tulu Bor does not crop out in area 131. In our Fig. 3 of ref. 1 we emphasised this by

not showing the tuff in the stratigraphic column and by stating in the caption, 'The Tulu Bor Tuff is believed to be located approximately 6 m below the base of this section.' This statement was based on Fig. 2 of Leakey⁴, which infers the location of the Tulu Bor in area 131. Concerning the observation that the results from areas 15 and 129 are not from the same magnetostratigraphic column, Harris may have missed our comment, "'Area 129' column is a composite: the upper portion was from area 129, the lower from the adjoining area 15." Finally, we note that Harris's comment that there is no need to postulate secondary magnetisation for the early part of the area 102 succession is in accord with the following statement in our article: "Identities of tuffs are unknown in the lower portion of area 102 column, so we can no longer assume that this section was overprinted with normal polarity."

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Maya dates AD 352–1296

PLANETARY conjunctions, in my recent article¹, were used to fix the floating chronology of the Classic Maya codices and stelae. Many planetary configurations, however, repeat themselves after 59.1 yr so that multiple solutions are possible. I first supposed as a limiting condition that the conventional (Goodman–Martinez–Thompson) dates were in error by not more than 30,000 d, so postponing investigation of a possible solution 1,541 days beyond that limit. I found that an adjustment by 27.3 yr brought the planetary cycle into phase, and the adjacent solution 59.1 yr or 21,574 d greater was not fully investigated. Either solution would fit the conjunctions of AD 650/3, 709/11 and 770. My present work², has shown that it was my first solution that was spurious: 615,824 d must now be added to all Mayan Day Numbers to get Julian dates, and 59 yr 24 ± 1 d must be added to the Maya dates in my previous^{1, 3} articles. This is 86.4 (86 yr 4 months and about 3 d) yr or 31,541 d greater than the conventional dates.

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reviews

Science as a never-ending quest

J. R. Ravetz

The Nature of Science: The History of Science in Western Culture Since 1600. By David Knight. Pp. 215. (André Deutsch: London, 1977.) £4.95.

In comparison with many other disciplines studying human behaviour, the history of science is quite peaceful and self-confident. Although divergences of approach have always existed, they have never polarised the whole field. The present book represents a considerable shift in the consensus of what the history of science is all about, and yet it is done in a relaxed, matter-of-fact way, totally devoid of programmatic pronouncements.

What David Knight argues, in *The Nature of Science*, is that it lies as much in the activity of research and its context, as in the results we accept as valid. The supposed 'hard core' of objective facts, which could be studied on their own by philosophers and historians, is now blended into the matrix—social and intellectual—of scientific work.

In order to exhibit this view of science, David Knight must approach history in an unorthodox way. Instead of treating 'periods' in chronological sequence, he uses 'themes'. We have a progression, from the more philosophical ("Science as a Complex Activity", and "... as Explanation"), to the more social, linking science to its community, and discussing careers, Government and utility. The bridge-section is on "Science and Not Science".

It is very refreshing for a person familiar with the history of science to see the materials sorted in this way. But this arrangement has its costs, most notably in the loss of a chronological sense for the whole story.

The general literary style is relaxed and conversational. The book makes enjoyable reading, but the shape of events in history becomes quite blurred as the narrative slides from one topic to another.

The history that is told in such a way, especially in a small book covering such a wide range, inevitably becomes a personal statement. The materials reflect the author's own interests; here, social and institutional aspects of English science absorb much space. Also, the text is liberally sprinkled with the author's own generalisations and morals. They are

recognisable as such, and fit with Dr Knight's commitment to the view that history, like natural science, is a never-ending quest, best not left exclusively with professionals.

Everyone will find fault or favour with his/her own selection of wisdom. I am intrigued by the suggestion that eighteenth-century chemistry was hindered by the Newtonian orthodoxy; this is a far cry from the classic ridicule of 'phlogiston' as the nonsense theory that awaited overthrow by Lavoisier. Some throwaway statements have

rather strong implications; to say that conservatives were "naturally enough often right in resisting innovations", opens up new and challenging perspectives on the history of scientific revolutions and indeed on the methodology of science.

So here is a book to be enjoyed rather than dissected; a very useful introduction to a growing new historiography of science. □

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Genetic expression in plants

Molecular Aspects of Gene Expression in Plants. Edited by J. A. Bryant. Pp. 338. (Academic: London, New York and San Francisco, 1977.) £9.60; \$21.

THIS seven-chapter volume has been compiled by four authors, one of whom is the editor. Together they have many years experience with plant nucleic acids, and they have pooled their joint approaches and expertise to produce an overall view in what the dust cover calls "the first book to deal specifically with gene expression in plants".

The first half is a mine of basic information on the properties and functions of DNA, RNA and proteins in cells, and freely uses information from non-plant sources. The first chapter on nuclear DNA deals effectively with the extraction and properties of DNA, the structure of chromosomes and the modifications and variations that can occur in the genome following synthesis and turnover of DNA *in vivo*. Bryant has done a service in confining the term "nucleohistone" for the DNA-histone complex and "chromatin" for the DNA-histone-acidic protein complex throughout the volume. Although the classification given for histones is the "f" nomenclature, it would have been helpful to undergraduate readers if the "H" nomenclature, which they will surely encounter in the literature, had been included. Nucleosomes receive brief mention only, and although buoyant density fractionations of DNA are discussed, the use of sucrose density fractionations is not.

The chapter on RNA structure and metabolism provides a lucid account of the different classes of RNA, outlining their separation and function as intermediates for the translation of genetic information in protein synthesis. Considerable space is given to the topic of precursor RNA molecules and their processing to ribosomal RNA in both prokaryotic and eukaryotic plants. The possible role of tRNA molecules and the stability and function of mRNA are discussed in relation to well studied examples of the control of transcriptional and translational events in plants.

There follows a straightforward account of the translation steps in protein synthesis with frequent comparisons with bacterial and mammalian systems. Most of the plant information has been gleaned from the extensive work on germinating seeds and protein-synthesising cell-free systems from wheat-germ. Attention has properly been drawn to the gaps in our knowledge for plants. The diagrams and schemes are somewhat repetitious, but the points are very clearly made.

A useful chapter is devoted to gene expression in chloroplasts and mitochondria, to include the basis of their role in cytoplasmic inheritance and the degree of their developmental autonomy. The nature and replication of chloroplast DNA, the transcription of RNA and the assembly of chloroplast proteins (particularly fraction I protein), are presented in an interesting way. The section dealing with mitochondria is brief, but sets out present knowledge of their DNA structure and RNA transcription products, together

with the meagre information on their protein synthesis.

The factors linking DNA replication with cell division are treated at length in a wide-ranging and well presented chapter on the cell cycle. Attention is focussed on patterns of nuclear RNA and cytoplasmic protein synthesis and the modifications in chromatin that occur through the different phases of the cycle. Changes in enzyme activities that accompany these cyclic events are well presented. Attempts to isolate control factors in mitosis in plants are described and the role of cyclic AMP, though mentioned, is properly treated with caution. The overall information is neatly summarised and the reader will appreciate the comprehensive diagrammatic presentation of the known regulatory events occurring through the cell cycle of many eukaryotic and prokaryotic types, together with the leads into hormonal and nutrient gradients that can turn on and turn off division in plant cells. Although to some extent the conditions that trip the switches are known, the reader will learn that the operating mechanisms for the switching systems are little understood.

The chapter dealing with molecular aspects of differentiation rests uneasily within this volume, for although it describes in detail the ultrastructural morphogenesis of certain cell types it is not concerned directly with the mechanism of gene expression. It includes, however, a useful and well reasoned

survey of transcriptional, translational and post-translational control in protein synthesis.

In the final chapter on plant growth substances we learn that the term 'plant hormone' is going out of usage (which I doubt) and that these regulatory substances are the keys to our understanding of differentiation and organisation (with which I concur). Two examples of altered gene expression are considered in detail. One, the control by gibberellic and abscisic acids of the synthesis and secretion of hydrolytic enzymes (especially α -amylase) in barley aleurone cells, and secondly, the role of auxin in the control of cellulases during cell expansion. These aspects of differentiation are well integrated with the earlier chapters on the involvement of nucleic acids and proteins, and include discussion of the conclusions that may be drawn from hormone-modified changes in incorporation and enzymic activity studies.

Taken as a whole, the content of the book is stimulating and very readable. The bibliography and reference system are excellent, and the illustrations and tables have been carefully selected to be simple and instructive. This book is timely and relevant, and will be much used by those seeking information on genetic expression in plants. □

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Peasants to tractor drivers

Cognitive Development: Its Cultural and Social Foundations. By A. R. Luria. Pp. xvi+175. (Harvard University: Cambridge, Massachusetts, 1976.) £9.40.

THIS book reports a pioneering study done in the early 1930s by the gifted Russian psychologist, Luria, to determine the impact of collectivisation, literacy, and newly introduced technology on the peasants of remote Uzbekistan and Kirghizia. It looks for the impact on the cognitive and intellectual processes of those affected: their perception, categorising, reasoning, and self-image, in the conviction that these processes are not simply biologically given but are shaped by cultural-historical processes—a view now widely shared by Marxist and Western psychologists alike, though by no means so in the 1930s. Interestingly enough, the work did not see the light of day in book form in Russia until 1974, its publication having been 'discouraged' as disrespectful of minority group sensibilities when the results

were first reported at a time when official Soviet policy was strongly directed toward the integration of minority nationalities, shades of the American experience in the late 1960s with respect to the Blacks in the United States.

The point of view of the book is 'developmental' in the sense that the experience of schooling and modern technological 'collective' life is seen as 'completing' the stages of mental development of illiterate peasants, whatever the age at which they were exposed to the new influences. Although the methods used were 'clinical-experimental' procedures, involving various kinds of sorting and reasoning tests followed up by probing interviews to get at the basic reasons for subjects operating as they did, the results are quite clear at the factual level. Illiterate peasants tend to be much more concrete, function bound, 'complexive' (in the sense of thinking thematically rather than syllogistically), and tied to immediate experience than are their comperes who had been exposed to schooling and the cooperative-technical ways of the collective farm. Luria characterises the advance as 'the leap

from the sensory to the rational.' Even with respect to characterising themselves, the illiterate peasants have great difficulty achieving detachment, seeming quite unable to characterise personal traits in a fashion free from functions and duties: they are more situation bound than their literate brothers.

The issue that the book poses is one of interpretation. Granted that 'cultural-historical' factors are important, is it a question of different intellectual powers being available to the two groups on the basis of experience, or is it rather the ways in which human beings, using the same underlying powers, adapt intellectually to their circumstances? An example from the test of syllogistic reasoning illustrates:

LURIA: Cotton can't grow where it's cold, and it's cold in England. Does cotton grow there or not?

KHAMRAK (an illiterate miller from a remote village): If it's cold it won't grow, while if it's hot it will. From your words, I would have to say that cotton shouldn't grow there. But I would have to know what spring is like there, what kinds of nights they have.

Khamrak, to be sure, is distrustful of reasoning from *a priori* premises. But may it not reflect a deeper adaptation to a mode of life where skill and experience count more than in a technical society, such that knowing *that* is subordinated to knowing *how*?

The work of Cole and Scribner, of Horton, and of Labov, all indicate that cultural factors produce a different deployment of universal, basic cognitive and intellectual processes of different peoples, that these deployments do not on close inspection turn out to be any less 'rational' when applied to one set of circumstances than any other. Personally, I would rather trust Khamrak with local agricultural decisions than most of my Oxford undergraduates who could solve the cotton syllogism in a trice. Perhaps the fault lies with the use of a developmental model to account for differences produced by differing technical cultures.

Be that as it may, the book is rich in data and observation on the nature and transformation of the peasant mentality into the more familiar 'rational' reckoning of those who went on to run the tractor pools and marketing arrangement of the *kolkhoz* that transformed Soviet agriculture and life in the country. Luria brings a rare genius to the task, managing, as he has in his past books, to find the germ of generality in the everyday particulars to which he brings his observational skills.

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Biomolecular mixture

Intermolecular Interactions and Biomolecular Organisation. By A. J. Hopfinger. Pp. xviii+395. (Wiley Inter-Science: New York and London, 1977.) \$33; £19.50.

NEW results in science are published and then reviewed in journals specialising in broad or narrow topics of current interest. Books should be different in that they represent a new synthesis of fact and theory collected and distilled into a coherent and useful entity. Unfortunately, we are facing a deluge of books which offer very little that has not earlier been given in closely related form. The volume under review, although attractively titled, is a compilation of disjointed topics with no visible thread leading from one to the other. One cannot hope to learn from it about the theory of solutions of macromolecules, or conformational analysis by nuclear magnetic resonance, or quantum mechanical calculations, nor can one form a critical opinion about how these connect to the problem of drug design, for instance, to which a whole chapter is devoted. I found particularly

revealing the admission of the author that large portions of his book are indeed taken (p145 or p252, for instance) "from the excellent review" of other authors!

After trying to examine the extensive subject matter somewhat more closely, I am reminded of a story told by the late author, Maurice Samuel, in his quest to evaluate the widely ranging work of a famous contemporary historian. All fellow historians approached indeed acquiesced that his study of history seemed highly significant; however, as far as their own speciality was concerned, they voiced grave reservations. Trying to evaluate not only the breadth of the present work, but rather the depth to which individual topics are treated, particularly those with which I am familiar, I ran into similar difficulties. To mention a few examples only, our own work on hydration of DNA is incorrectly quoted (p160), lumped together with other quotes in purely irrelevant fashion. Concerning our work quoted in the footnote of Table 8-1, viscometry of DNA cannot be used directly to compute the number of intercalated dye molecules. On p265, part of the enormous Table 12-1, the author reprints an incorrect finding first made in 1958, and then often repeated, that glutamic

dehydrogenase is a zinc enzyme with a molecular weight of 1,000,000 containing two to six Zn molecules. For many years now it has been known that glutamic dehydrogenase has a molecular weight of six times 56,000 and does not contain Zn. The discussion on nucleohistone aggregation and chromatin structure in chapter 13 is largely outdated and would be unintelligible to the uninitiated. Space does not permit me to go into other examples, which abound.

The proof-reading is sloppy beyond the border of insult. Having myself recently published a book, I know how difficult it is to win the battle with publisher and printer. Yet one must try. The author is responsible that the same name is spelt Fahien, Fehiem, Fahiem on one page(152), Subivana and Subirama (the correct name is Subirana) on p204, and Schleich, Scheich and Schiech in chapter 12. There are many more such examples. A carefully proof-read name index would have been helpful in a work which draws on so many varied sources.

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Science policy yearbook

Science and Government Report International Almanac, 1977. Edited by Daniel S. Greenberg. Pp. 305. (Science and Government Report: Washington DC, 1977.) \$54 before 1 September; afterwards \$75.

THIS first volume in what is intended as an annual series introduces the idea of a 'science policy yearbook'. Part I consists of 20 chapters which survey, nation-by-nation, major recent developments in science and technology policy. Most authors are experienced science journalists (not a few of whose names will be well known to *Nature* readers), and the chapters are concise, informative and wholly unpolemical. Each covers more or less similar ground: changing priorities; research and development expenditure; changes in government research organisation and science policy advisory machinery; and issues which in the country concerned have been publicly contentious.

I personally enjoyed this part of the book and can recommend it to anyone who wants to keep broadly in touch with what is happening on the interface between science and government in a wide range of countries. Though more

readable than the products of international organisations active in this field, it lacks the systematic quantitative data which often makes their reports a useful basis for further analysis.

Part 2 of the book, an international directory of major scientific organisations and science policy personnel, though commendably up to date, is curiously haphazard in its coverage. Thus, although we find listed British Departmental Chief Scientists and Britain's Scientific Counsellors overseas, the only organisations whose addresses and functions are given are the Central Policy Review Staff in the Cabinet Office, the SRC, MRC and Department of Energy.

Part 3 gives excerpts from eight recent (mostly American) science policy documents, including President Carter's campaign speeches and the NIH guidelines on recombinant DNA experiments.

On balance, the yearbook idea is an interesting one, and libraries might wish to acquire this first effort notably for the contents of Part 1.

Stuart S. Blume

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**tins
will
change
your
reading
habits**

Early development and gene activity

Gene Activity in Early Development. Second edition. By Eric H. Davidson. Pp. xvi+452. (Academic: London and New York, 1977.) £11.30.

THIS is an enlarged and completely rewritten edition of Dr Davidson's successful 1968 book of the same title. It is the only comprehensive review, at the molecular level, of early animal development, and provides a scholarly and unbiased account of the field as it now stands. It will be invaluable to research scientists and advanced students interested in this field.

The book consists of eight chapters, covering the following topics; the constancy of the genome; the initiation of genomic control and of cell differences; the changing populations of proteins and RNAs in embryos; cytoplasmic determinants, and oogenesis. Each chapter is preceded by a summary of the principal facts. Within most chapters, results are presented according to taxonomic groups. This is a wise decision, because there are remarkable differences in the morphological and molecular characteristics of development of different animals, a situation which has made it impossible so far to identify any universal mechanism of development applicable to all animals. One chapter provides a very helpful explanation of renaturation and molecular hybridisation experiments, such as have been used in much of the author's own work. One of the particularly good characteristics of the first edition which has been retained is the survey of classical embryological literature. This is especially valuable since most of those engaged in the molecular analysis of early development have no familiarity with, or convenient access to, early literature of this type. A detailed and very representative bibliography of original papers and their titles, up to the middle of 1976, is provided.

The general theme which lies behind most of the work reviewed in this book is the importance of gene activity in development. The author's view is that during oogenesis (egg formation) gene products are accumulated. Soon after fertilisation, they are moved to different positions in the egg, and as a result, they (or other molecules formed by their activity) cause activation of different genes in different cells. These activated genes start to make their own products during gastrulation and at later stages. This represents a fairly conventional view of early development, having been favoured by many

writers since the end of the last century. Certainly the detailed evidence summarised in this book does a lot to bolster one's confidence in this hypothesis.

It is currently fashionable to devote much attention to cell surface molecules, and those who fall into this class may feel that this aspect of the molecular repertoire of cells is lightly represented. There is no evidence at present, however, that any of these molecules are causally connected with development. An area of relevant information which is not covered in this book concerns the molecular differences between highly specialised adult cells. It is possible that a detailed molecular description of differences between adult cells may lead more directly to an understanding of early developmental processes than a des-

cription of molecular changes which take place in embryos themselves.

As is evident from reading this book, two factors restrict progress in this field. One is the lack of a detailed description of the activity of single known genes; the other is the absence of a way of distinguishing genes and gene products which are important in development from those which merely accompany this process in a non-causal way. Procedures are becoming available by which these kinds of information can be obtained; but for many years to come, this book is likely to be a standard reference work for all those interested in early development and gene activity.

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Tilting at windmills

Ecological Sanity. By George Claus and Karen Bolander. Pp. xv+592. (David McKay: New York, 1977.) \$16.95.

It is difficult to understand why the David McKay Company of New York have bothered to publish this book in 1977. They very honestly admit in a note at the front that the research (*sic*) on which it was based was completed in 1972, and that it was delayed by "production difficulties beyond our control or that of the authors". The authors' Preface has a dateline "Vienna 1972". The publishers state that they are "confident that the continued significance of this book will be apparent to the reader". Unfortunately this does not apply to this reviewer.

Ecological Sanity sets out to give a critical examination of the statements made by the so-called "Ecological Lobby", particularly in the United States. The lobby is accused of putting forward statements which cannot be supported by sound research, and for making premature Doomsday announcements. This is all set out at great length, for the book is not far short of 300,000 words. Had it appeared in 1972 it might have been of some interest, although even by then many other authors had already produced more effective criticisms of the errors put forward by the more extreme environmentalists who had assumed the title of "the ecologists". It is true that some of these extremists are still unrepentant, although many have either recanted or disappeared from the scene. Claus and Bolander are, for the most part, tilting at windmills, for the situation is very different

from that which obtained five years ago. A far greater number of people, particularly those in authority, are able to view the scene more rationally. Thus, the World Health Organisation still recommends DDT for malaria control in appropriate situations, although the dangers of misuse are fully recognised. The more absurd statements of the lobby have been recognised as such; their other efforts to keep the rest of us on our toes, is probably a good thing.

There has, of course, been controversy about pesticides, particularly the organochlorine insecticides. The 275 pages of small print (almost half the book) on this subject is particularly unsatisfactory. It is true that, in the late 1960s and early 1970s, some environmentalists extrapolated unjustifiably from the results of research. There has, however, been a great deal more work done in the past five years. Some of this disproves conclusions based on extrapolations, some supports conclusions hitherto unproven. This omission of what is now the major part of the relevant research means that the book is of interest only to the historian of the bye-ways of science.

Yet there is still a need for us to be reminded of the need for ecological sanity, even if this book does little to achieve this objective. Environmental dangers still exist, and it is as bad to exaggerate their importance as to refuse to recognise their existence. Resources will always be limited, and environmental protection will always be subject to compromise. This is unfortunately not as glamorous as extremism from the "right" or from the "left".

Kenneth Mellanby

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obituary

Sir Graham Sutton

SIR GRAHAM SUTTON, former Director-General of the Meteorological Office, who died on 26 May 1977 at the age of 74, achieved eminence early in his career by fundamental contributions to the theory of atmospheric turbulence and diffusion.

His work in this field began in the late 1920s, when atmospheric boundary-layer theory was at a primitive stage. It had, however, become evident, from early observations of diffusion and from pioneering theoretical studies by G. I. Taylor and L. F. Richardson, that the diffusion of a plume of smoke could not be explained on the basis of a constant eddy diffusivity analogous to, but very much larger than, the molecular diffusivity of kinetic theory. The starting point of Sutton's theory of turbulent transfer was the combination of Taylor's famous theorem, for the kinetics of passive particles carried in a turbulent fluid, with the classical Prandtl mixing-length theory. This led to an explicit height-dependent form of the eddy diffusivity. It also led to the result that the spread of a plume of gas or fine particles issuing from a point in a wind was proportional to distance raised to a power between $\frac{1}{2}$ and 1, the precise magnitude of this exponent and of the proportionality constant being determined by the form of the increase of wind with height above ground.

The consequences of this work have been wide-ranging and long-lasting. It provided the basis for the first formulae representing the effect of meteorological conditions on the concentration-field downwind of a source of gas or particles, and Sutton's point-source formulae, at first in restricted use in chemical warfare studies, became widely known immediately after the 1939–45 war in the context of industrial air pollution. It also was a stimulus for continuing and far-reaching boundary-layer studies, notably of the wind profile, the aerodynamic drag of the earth's surface and the factors determining evaporation from water surfaces and transpiration from vegetation.

Oliver Graham Sutton was born in Cwmcarn, Monmouthshire, in 1903. He took his degrees in mathematics at Aberystwyth and Oxford and his first appointment was to a lectureship at Aberystwyth. Two years later, in 1928, he was persuaded by the late Sir David

Clair-Continental

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Brunt to join the Meteorological Office and shortly afterwards he was seconded to the War Office establishment at Porton, where the aforementioned work was carried out.

In 1942, with his potential for scientific administration already strongly evident, he was required to leave his meteorological team for wider war-time responsibilities as Superintendent of Research at Porton, and he was drawn into further fields of administration in posts as Superintendent of the Tank Armament Research Station (1943–45) and Chief Superintendent of the Radio Research and Development Establishment (1945–47).

In 1947 he returned to individual scientific work in the position of Bashforth Professor of Mathematical Physics at the Royal Military College of Science, Shrivenham, though here also wider responsibilities were to be placed on him, as Scientific Adviser to the Army Council (1951) and Dean of the Royal Military College (1952–53). His individual scientific achievements were recognised in his election as a Fellow of the Royal Society in 1949.

During this phase of his career he was able to turn to more general writing on the fundamental science of boundary-layer meteorology, an ambition on which he had made a start while at Porton. His first published effort was *Atmospheric Turbulence* in the Methuen Monograph series (1949) and this was followed by his well-known major work *Micrometeorology*

(1953), in which he covered the most important topics in the physics of the surface air layers. Although much had already been written on the qualitative aspects of those topics, *Micrometeorology* was the first connected account of the relevant principles and crucial observations in a full mathematical-physical setting. His capacity for exact mathematical analysis and lucid exposition of complex physical interpretations came out here to the full, and he was to make good use of the latter gift over more than two decades of writing for less specialised readers, as for example in his *Science of Flight* (1950) and *The Weather* (1974).

In 1953 Dr Sutton accepted an invitation to become Director-General of the Meteorological Office, and for the next twelve years devoted his mature powers of leadership, scientific judgement and administrative skill to the formidable task of guiding its developing activities. His period of office saw the emergence of a fully-developed research division with the scope and reputation of a leading institute of meteorology. Technical resources were greatly expanded and included the installation of high-speed computing facilities. The services which meteorological science could usefully offer were extended far beyond those originally designed for weather forecasting and aviation. At the same time the hitherto scattered main branches of the Office were brought together in the present unified headquarters at Bracknell.

Sir Graham (he was knighted in 1955) planned to retire from professional activities in 1965, but he was then persuaded to be first chairman of the Natural Environment Research Council. Here again his outstanding abilities as an administrator and his qualities of leadership were turned to an urgent and important task, that of ensuring the rapid and successful development of the new organisation for promoting the various sciences of the natural environment.

The many recognitions and awards received by Sir Graham included the Symons Memorial Gold Medal of the Royal Meteorological Society (1959), the International Meteorological Organization Prize (1968) and the Frank A. Chambers Award of the United States Air Pollution Control Association (1968). There were also countless demands on his time for work on com-

mittees and in other organisations, and to many spells of service as chairman he invariably brought a balanced judgement, a diplomatic touch and a quiet sense of humour. While Director-General of the Meteorological Office he took a prominent part in the activities of the World Meteorological Organisation, especially as a member of its Executive Committee. He was President of the Royal Meteorological Society 1953–55. He also found time for the duties of a Justice of the Peace and for those of a member of the Council of the University College of Wales, Aberystwyth, of which he was Vice-President in his last few years.

F. Pasquill

A. D. Walsh

PROFESSOR A. DONALD WALSH FRS died in Dundee, after a long illness, on 23 April 1977 in his sixty-first year. He was educated at Loughborough Grammar School and Corpus Christi College, Cambridge, where he was a Mawson Scholar. In 1938 he started research in vacuum ultra-violet spectroscopy under the direction of Professor W. C. Price and continued as an ICI Research Fellow until 1949, when he accepted a lectureship at Leeds University. In 1953 he was promoted to a Readership, and two years later was invited to take the Baxter Chair of Chemistry at Queen's College, Dundee, which was then part of the University of St. Andrews. In 1967, when Queen's College became the University of Dundee, Professor Walsh was appointed Dean of the Science Faculty. He was elected to the Royal Society of Edinburgh in 1959 and to the Royal Society in 1964.

Donald Walsh will always be remembered for his enthusiasm. Whether he was lecturing to a first-year class, discussing his latest research, or watching the flight of a bird while driving erratically along a country road, he radiated a fascinating and infectious enthusiasm for his subject.

It was this enthusiasm which marked his rise to the heights of his profession. The investigation of the electronic structure of small molecules and the nature of valence and chemical bonding were, for him, an absorbing study throughout his career. The topic suited his enquiring mind ideally, for he loved to put his ideas to the test and spent very many happy days fitting together his theories and his experimental spectra.

During the war, when it became necessary for research to have more practical application, Donald Walsh became involved in problems of gaseous combustion, such as the suppression of the exhaust flames which

revealed the presence of our aeroplanes in the sky at night. After the war, he became more interested in the mechanism of gaseous combustion reactions, and in particular with the mechanism of anti-knocks in petrol engines. In this he was associated with Riccardo Ltd and later with Octel Ltd who still employ a number of his former students. Those privileged to hear him present his research papers in the 1950s and 1960s will clearly recall the rival theories on anti-knock action put forward by Professors R. G. W. Norrish and A. D. Walsh, the debates being enlivened by the completely different personalities of these two eminent scientists. Donald Walsh was fond of simple experiments to demonstrate his ideas. One remembers the hydrogen peroxide which usually hit the ceiling in a demonstration that peroxides are rapidly decomposed by particulate lead oxide (an important part of his theory of the anti-knock action of lead alkyls.)

In Dundee his gentlemanly charm and hospitality quickly gained for him the loyalty of a department which rapidly grew under his direction and enthusiasm. His research students enjoyed many happy Saturday afternoons helping him construct his large garden, and they appreciated the generous quantities of Elin Walsh's magnificent teas which contrasted so markedly with student fare.

When Dundee gained independent status as a university in 1967, Donald Walsh was the obvious choice for Dean of the Faculty of Science—a Faculty which might have disappeared earlier but for his efforts. Extra duties did not deter him, and he habitually arrived in the Department at 6 a.m. after an overnight journey from London—he was still there at 7 p.m.

He bore his long fight with Parkinson's disease with typical fortitude as he was gradually forced to retire from chemistry and from the department to which he had devoted so much of his time and energy.

*D. E. Hoare
P. A. Warsop*

R. D. Brauer

RICHARD DAGOBERT BRAUER, who was one of the leading pure mathematicians of this century, died on 17 April 1977 at Belmont, Massachusetts. Born in Berlin in 1901, he studied at the university there under the distinguished algebraist Issai Schur. He taught at Königsberg until the political oppression in Germany forced him to leave in 1933. He held temporary posts at the University of Kentucky and the Institute for Advanced Study in Princeton. In 1935 he went to the University of Toronto, where he stayed until

1948; he then returned to the United States, and held chairs at the Universities of Michigan (1948–1952) and Harvard (1952–1971).

Brauer's work on the representation theory of groups has been a major influence in the development of algebra during the past half-century. Some of his earlier papers were concerned with the representations of semisimple continuous groups—Hermann Weyl's fundamental work on this subject appeared at about the time (1925–1926) when Brauer was presenting his doctoral thesis. Brauer's methods were more algebraic, and in some ways simpler than Weyl's. In fact Brauer worked as assistant to Weyl in Princeton in the year 1934–1935, and they wrote there a joint paper on spinors, which is the classical exposition of the mathematical background to Dirac's theory of the spinning electron.

From 1935 onwards, Brauer was increasingly concerned with the representation theory of finite groups. He developed a beautiful and deep theory of modular characters, which gave a completely new insight into the study of group characters. Group characters had been introduced in 1896 by Frobenius, and had already given, in the first decade of this century, some remarkable results on the structure of finite groups. It was Brauer who, thirty years later, resumed this work, and by the end of the 1950s had laid the foundation of a method for attacking the very difficult problem of the classification of finite simple groups. His pioneering work was one of the starting points of a great advance in this problem. Brauer's active involvement in this research continued right up to the end of his life.

During his long career Richard Brauer received many academic honours and held some of the highest positions in his profession. But he retained a very gentle and unassuming manner, and a concern for the problems and interests of other mathematicians. His modesty about his own great achievements was proverbial—a mathematical conversation with him would usually become a conversation about the other person's work. A first impression of unworldliness was deceptive; he used his time very efficiently, and achieved a great deal in an unhurried manner. He became a kind of elder statesman among American algebraists, and his advice was sought and valued for its wisdom and humanity.

He is survived by his wife Ilse (née Karger) whom he married in 1925; also by his brother Alfred, and his two sons George and Fred, who are all mathematicians.

J. A. Green

what's new—chromatographs

In addition to the regular Newly on the Market section in *Nature*, special features on a particular type of laboratory equipment or instrumentation will be published in this and forthcoming issues. The issue of 20 October will be on Spectrometers and ancillary equipment.

The purpose of a special feature is not to give listings of every item in the range of the manufacturers mentioned but to take a variety of equipment of interest in that feature area and give an idea of what kinds of instruments are available and from whom. There is a Reader Enquiry Coupon facing the inside back cover.

EDT Research now have a demonstration Jobin-Yvon Chromatospac preparative liquid chromatograph in the UK. This instrument has a loading capacity of 100 g of sample per injection and can provide high performance (typically 7,000 theoretical plates per m with TLC grade silica). Many different types of stationary phase have been used successfully including alumina and silanised silica for reverse phase. A unique pressure packing system for the stationary phase provides excellent reproducibility. The instrument also has the capacity to operate in the partial elution mode, as the stationary phase can be completely removed from the cylinder.

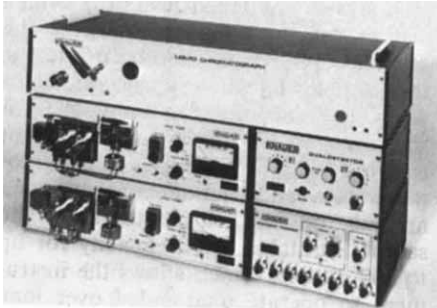
Circle No. 37 on Reader Enquiry Coupon

Phase Separations Ltd present HPLC adsorbents. Spherisorb is a range of highly efficient, totally porous, particles for HPLC and the range of materials is based upon 5 μm , 10 μm and 20 μm particles of alumina and silica with a variety of chemically bonded phases for reverse phase chromatography. The Model 1203 from Laboratory Data Control is a very sensitive ultraviolet detector designed specifically for use in HPLC and has selectable ranges from 0.002 to 2.048 a.u.s. The new detector has a noise level (typical short term) of 2×10^{-5} a.u. (P to P). A new range of glass capillary columns is now available. Both wall coated open tubular and support coated open tubular are available with a wide variety of stationary phases.

Circle No. 38 on Reader Enquiry Coupon

Knauer present a range of equipment: a differential refractometer for analytical and preparative use; a high temperature refractometer up to 160 °C; ultraviolet filter photometer (254 nm); a spectral photometer (200–600 nm); uv/ri dual detector; a double filter photometer (254–600 nm); a fluorescence detector; combination ultraviolet and fluorescence detector and conductivity detector. Knauer columns have specific advantages, namely, the inside diameter of the stainless steel column is especially honed to provide a highly polished surface and precise concentricity; symmetrical construction makes 'backflush' possible; the column ends have integral machined threaded terminations for safety. A replaceable seal is used instead of a ferrule, so columns can always be refilled. Stainless steel sieves and a fibreglass filter are used to retain all particulates larger than 2 μm . Knauer HPLC columns are modular in construction. The optimum separations obtained with analytical columns can also be expected with the preparative columns. The preparative columns (16 mm i.d., length 25 cm and 50 cm) may be packed with the same finely granulated packing materials as the analytical columns (4, 6 mm i.d.). The use of a 'stream distributor' in the column, produced a uniform spread of the sample onto the surface of the column and low dead-volume elute flow.

Circle No. 39 on Reader Enquiry Coupon



Knauer Dualdetektor

Rofin announce the Chromatix KMX-6, a revolutionary low angle light scattering photometer which utilises the spatial characteristics of a small, single transverse mode laser. The patented design allows measurement of scattering at forward angles of less than 2° from the incident beam, and permits weight-average molecular weights to be obtained on an absolute

basis in minutes, without recourse to an arbitrary standard. At such low angles, weight-average molecular weights may be calculated from scattering measurements at a single concentration and angle, if the solute second virial coefficient is known. When the second virial coefficient has not been determined, the data need only be extrapolated to zero concentration by making measurements at two or more concentrations. no angular extrapolation is required. Rapid instrumental response makes high speed kinetic measurements possible, and when combined with a computer and gel permeation chromatograph, molecular weight distributions can be determined. The high sensitivity and many unique design features of the KMX-6 permit it to be applied to a variety of applications in many different scientific fields, such as molecular weight determinations, molecular weight distribution (with GPC), macromolecular kinetics (enzyme and antibody-antigen reactions, dimerisation, etc.), single particle fluorescence and nephelometry, molecular interactions (virial coefficients), molecular conformation (radius of gyration), molecular anisotropy (depolarisation), Rayleigh spectroscopy (diffusion coefficients), Brillouin spectroscopy (acoustic modulation), and refractometry.

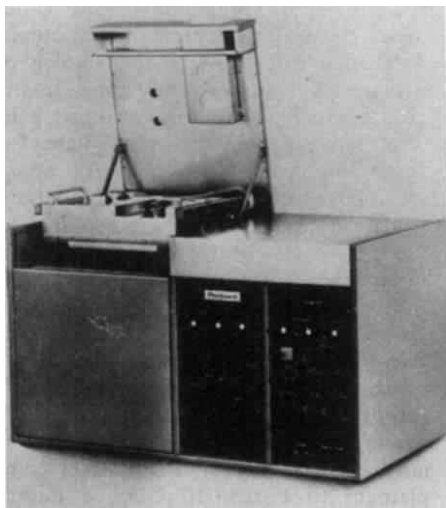
Circle No. 40 on Reader Enquiry Coupon

Whatman supply a vast range of chromatography columns and packings and modern liquid chromatography of nucleic acid constituents and other other naturally occurring compounds. Whatman offers the widest choice of prepacked columns and loose media commercially available. Silica gels, aluminas, polyamide, bonded phases for reversed-phase and normal phase chromatography are available in both prepacked, pretested columns and as loose media. Many of these have direct application in HPLC for separation of different classes of biologically significant compounds, e.g., steroids may be separated on Partisil. Various types of Partisil appropriate for different types of chromatography are available e.g., Partisil-10 PAC, for normal phase partition, HP2C, a complement to Partisil-10 ODS, a micro-particle non-partition bonded phase for

reverse-phase HPLC and so on. Both pellicular and microparticulate media are available. The microparticle-support bonded ion exchangers are increasingly important ion exchange media because of higher efficiencies, greater loading capacity and other characteristics. Nevertheless the pellicular forms will continue to have an important role since packing of columns with microparticulates in the laboratory requires experience and apparatus not commonly available; in contrast ion exchangers in pellicular form are easily dry packed. Further, microparticle packings perform optimally when the chromatographer is experienced enough to be able to fully utilise their inherent superiority. In general, then, pelliculars are used until the techniques of modern liquid chromatography are mastered. One benefit of pellicular ion exchangers is that they are relatively unaffected by sample contamination. Should the pellicular column become inoperable due to impurities in the sample, the contaminated portion of the column bed may be removed and replaced with new media. **Circle No. 41 on Reader Enquiry Coupon**

Packard introduce the 428 Series gas chromatographs which combines requirements for accuracy and reproducibility with unique analytical and instrumental flexibility. The analytical flexibility is provided through the column oven cover which contains dual injection ports, dual detectors and columns. This analytical module is a plug-in unit, thus providing the possibility to change the complete GC analysis system in minutes. Instrumental flexibility is achieved by the use of plug-in electronic modules and plug-in flow control units. Any dual detector configuration is possible, eg, dual FID, dual ECD, ECD/FID, TCD/FID etc.

Circle No. 42 on Reader Enquiry Coupon



Packard 42F Chromatograph

Pye Unicam present a wide range of general and high performance chromatography equipment. The analyst involved in repetitive routine gas chromatography analyses requires a reliable low cost, compact workhorse to achieve a steady throughput of results. The GCD chromatographs are to meet this demand.

Typical applications for the GCD are to be found in laboratories engaged in multiple analyses such as quality control in the oil and gas industry, air pollution measurement and routine screening for drugs and hormones in clinical chemistry. These new flame ionisation chromatographs incorporate dual heated injection ports and dual



Pye Unicam LC3

heated flame ionisation detectors for dual column operation. Temperature control of the column oven may be chosen from isothermal or temperature programming; with conversion from isothermal to temperature programme being able to be carried out at any time. The basic chromatograph's also include a wide range ionisation amplifier, separate injector and detector oven temperature setting controls, and readout of temperature from the injectors, column oven and detector oven. The most useful accessory is the Autoinjector S8 which completely automates measurement of sample volume and injection of liquid samples. It is a self contained accessory which utilises punched tape to control the sequence of events that occur during the sample injection cycle. Standard tapes give facilities for six washes of the syringe before injection solvent washing of the syringe, control of integrators and identification of each analysis on the chromatogram. The sample turntable has a capacity for up to 100 samples which allows the instrument to operate unattended over long periods of time. A built-in interval timer determines the period between analyses, and may be set between the limits 0-120 min. The new Series 204 has modular construction providing a complete range of analytical gas chromatographs, starting from a simple instrument which can be subsequently extended to a fully automatic system. The basic oven assembly is suitable for single or dual column operation

and includes injector, detector and column ovens all with temperature readout. A choice of five detectors is available, any two of which may be operated simultaneously. The Autoinjector 8 can also be integrated. The new Series 604 process chromatograph system is also geared to the less complex applications and extensions up to six streams and six components can be handled with ease and economy, due to modular extension for sample handling, analysis and programming. **Circle No. 43 on Reader Enquiry Coupon**

The Dickinson Instrument Company announces a Linear Gradient Maker for column chromatography and gel electrophoresis. The linear concentration gradient maker has been designed for use in column chromatography and gel electrophoresis. Mixing is accomplished by placing the gradient maker on a magnetic stirrer. Out flow is by gravity or peristaltic pump. The apparatus has a unique, leak proof valve which provides easy access, positive on-off control, with no cross hole to line up. 1,200,600 or 300 ml sizes.

Circle No. 44 on Reader Enquiry Coupon

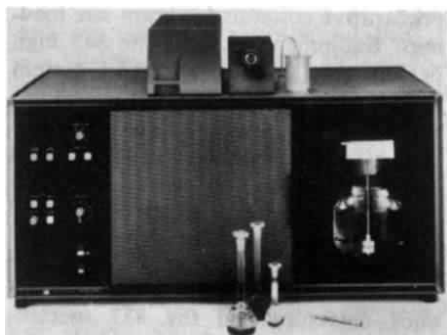
Tracor introduces a new detector, the third generation nitrogen-phosphorous GC detector. Model 702 which combines unique mechanical design with latest electronic technology to provide burnout protection for the alkali source. An exclusive desensitising mode allows routine use of chlorinated solvents and derivitising reagents not possible with previous designs. Tracor's Model 970 Variable Wavelength Detector is developed specifically for use with high pressure liquid chromatographs. This extends the range of standard absorption detectors from one or two discrete monitoring wavelengths to the complete ultraviolet and visible spectral range. This provides additional LC measuring capability for materials which do not absorb electromagnetic radiation at the previous standard wavelengths and enhances sensitivity for those materials which have high absorptivities at other than standard wavelengths.

Circle No. 45 on Reader Enquiry Coupon



Tracor 702

Hewlett-Packard offer a new compact high performance liquid chromatograph, the HP 1081A which performs basic LC functions with an accuracy and repeatability similar to that of other members of the HP 1080 family of liquid chromatographs, but at a fraction of the price of the top-of-the-line HP liquid chromatograph. The heart of the instrument's virtually pulseless flow system is a single-head diaphragm pump whose strokes can be adjusted by a pushbutton-controlled stepping motor to set the solvent flow. The pump design assures excellent flow stability. The selected pump-stroke position and the resulting column pressure (bar) are shown on the unit's three-digit numeric display. A valve



Hewlett-Packard 1081A

loop injector, allowing sample introduction at full column pressure without interrupting flow, comes standard with a 20- μ l loop, but 10-, 50-, and 100- μ l loops are also available. Other features of the flow system include a 2-l solvent reservoir with purge-gas connection and a column compartment that, although not heated, provides a protected column environment. The 1081A has a single wavelength UV detector with high signal-to-noise ratio that operates at 254 nm. The instrument, with its analog-signal output, can be linked directly to conventional integrators and strip-chart recorders. Interfacing the 1081A to a computer-based laboratory automation system is possible when an A to D converter is added. The HP 1080 Series LC family concept includes downward compatibility. Analytical methods within the capability of the new 1081A can be developed on the larger, processor-based HP 1084A, the most powerful LC of the HP product line. The new methods, then, can be transferred for routine work to the smaller HP 1081A with comparable precision. The HP 5985A, with dual chemical/electron ionisation source, provides greater flexibility in measurement and data handling with such advanced features as automatic tuning, an efficient hyperbolic quadrupole mass filter, a microprocessor-controlled GC, a powerful HP 21MX E Series computer and all-

digital electronics. Mass range is 10-1,000 amu and sensitivity is to picogram levels, essential for the analysis of pesticide pollution, drug and biological samples. The instrument's new inlet system is specifically designed for both packed and capillary column operation in either CI or EI mode. Sample injections may be split in a variable ratio between the GC and MS detectors to provide simultaneous quantitative GC analysis and positive identification of mass spectra in the same run. The gas chromatograph used in the system is a second-generation, microprocessor-controlled HP 5840A. This dual-column GC can be equipped with multiple detectors including dual-flame ionisation, dual-thermal-conductivity, flame-photo-metric, nitrogen-phosphorus and electron-capture detectors.

Circle No. 46 on Reader Enquiry Coupon.

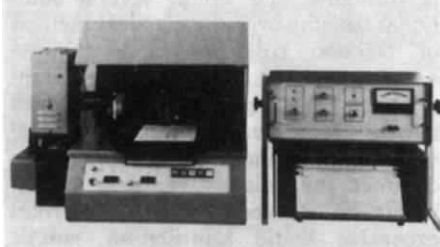
Coulter Electronics Ltd introduce the Model 725 automatic injector for use with high performance liquid chromatography systems. In use it allows the automatic, unattended injection of up to 64 consecutive samples with negligible carryover between samples. The unit is microprocessor operated and it controls the number of sample injections (up to 3 per vial), injection time, rinse between sample (if desired) and automatic shut-down after completion of last sample or detection of an instrument malfunction. It uses inexpensive disposable glass vials with a unique feature. The vials caps act as a piston to discharge sample with a positive flow. Only 700 μ l of sample is required and the injection valves will operate at pressures up to 6,000 p.s.i. and a 10 μ l sample loop is fitted as a standard. The unit can be used with any HPLC system and it can be controlled by a computer or integrator.

Circle No. 47 on Reader Enquiry Coupon

Schoeffel introduce a new and improved, extremely-sensitive continuously variable wavelength fluorescence detector for liquid chromatography. While using standard tested fluorometric technique, this new instrument offers advanced design features necessary for obtaining sensitivities required for measurements of minute fractions achieved by the superior separation capability of HPLC. Extreme care was taken to prevent fraction mixing and dead volume influence, and a unique to be efficiently collected from rather dead volume influence, and a unique 2 π steradian cuvette design (5 μ l) resulted. This development enables light to be efficiently collected from rather weak fluorescing signals emitted from minute sample quantities (typical sample size in picogram range). The FS 970

uses a broad band deuterium (or tungsten) lamp with a high throughput monochromator to enable the user to work at the compound's absorption maximum and to assure that a wide and variable spectral range is usable. This offers the investigator greater versatility since compounds can often be excited at several wavelength. The fluorescence emission measurement system consists of a superior end-on, state of the art photomultiplier tube and a solid state photometer to offer maximum sensitivity.

Circle No. 48 on Reader Enquiry Coupon.



Schoeffel SDC300/3000

ESI Nuclear introduce a new universal radioactivity detector for gas chromatography. Light and compact, the advanced design of the new system has resulted in easy, clear controls highly suitable for the less experienced laboratory personnel. It is of great use to those involved in drug and pesticide metabolism, research or similar fields. ¹⁴C labelled effluent from a gas chromatograph is passed through a furnace containing copper oxide. The ¹⁴C is oxidised, passed over a drying agent, and finally counted in a gas-flow proportional counter to determine the radioactive content. Tritium compounds may also be analysed in this way by the use of an additional reduction stage containing iron filings. The new universal 505 system has a flexible heated stainless steel tube which is temperature controlled to prevent condensation of the sample in the tube connecting the gas chromatograph to the radioactivity detector.

Circle No. 49 on Reader Enquiry Coupon

Lignes Télégraphiques et Téléphoniques present microprocessor computing-integrators ICAP 10 and ICAP 5 for gas and liquid chromatography and amino-acid analysers. The two main functions are chromatogram processing and concentrations computing. They permit flexible measurement of the area of sophisticated peaks from modern chromatographs and measure, compute and visualise absolute or relative retention time. Also the micro-computing part of the systems allows the concentration calculation after introduction of response factors of each component either simple standardisation or according to an internal or external standard.

Circle No. 50 on Reader Enquiry Coupon

Laboratory Impex Ltd offers specialist equipment for the monitoring of radioisotope tracers used in both liquid and gas chromatographs. The company brings together the ICN Instruments Coruflow high performance liquid scintillation detector head and its locally manufactured counting electronics and accessories to provide extremely sensitive systems for effluents containing ^{14}C , Tritium and other beta radiation emitters. The use of continuous flow monitors for radio tracers with HPLC has been made possible by improved flow cell design and detector performance and brings several additional benefits over the classical method of fraction collection and separate sample counting. The most important are that (1) sample material is almost always 100% recoverable (cf normal scintillation counting); (2) results are obtained immediately enabling experiments to proceed faster; (3) frees expensive liquid scintillation sample changers for other work.

Circle No. 51 on Reader Enquiry Coupon

Erba Science (UK) Ltd announce a series of gas chromatographs, particularly suited for capillary column applications. The current range includes: Model 2151, a well established, reliable instrument which comes complete with a FID, electrometer, oven temperature controls, carrier and auxiliary gas controls, temperature programmer, Grob splitless injector and 20 m Jaeggi column Model 2351, the dual column version of Model 2151 which, in addition to the units and service outlined above, comes complete with constant flow controllers for each of the carrier gas lines, plus independent pressure regulators and pressure gauges for each of the supplies to the two detectors. Model 2900 is the newly introduced capillary dedicated gas chromatograph which may be used with split or splitless sample injectors, single or dual columns and a range of detectors. The standard specification incorporates a fast response electrometer, a large volume column oven with drop down door, digital temperature settings with readout on five points, right and left hand connections for a mass spectrometer and a heat exchanger for sub ambient operation. One of the main features of the Carlo Erba range of gas chromatographs related to capillary column work, is the lack of dead volume. This is due to an on column injection system and a unique column detector coupling which allows the column outlet to be positioned at the detector jet.

Circle No. 52 on Reader Enquiry Coupon

Techmation present, for the random access analysis of refinery gas the automatic Series-S AGC which provides multiple access analysis of the hydrocarbons and inerts of interest in various types of refinery gases. The choice of two sample valve entry points for introducing samples enables determination of components of particular or immediate interest 'out of turn' from the order dictated by a single sample sequential analysis. Four columns are used in conjunction with two multi-function gas sampling valves and a function gas sampling valves and a column switching valve. Also included for sensitive, linear measurement of hydrogen while retaining sensitivity for other components is Carle's Hydrogen Transfer System (HTS). All valve switching is, of course, automatic.

Circle No. 53 on Reader Enquiry Coupon



Erba Science IIIH

Pharmacia Fine Chemicals present a new range of high performance columns and accessories for liquid chromatography in the life sciences. All components are solvent resistant and operate at pressures up to 10 kg cm^{-2} (140 lbs per square inch). They are autoclavable and fully compatible with biological substances. The system includes columns SR 10/50 J and SR 10/50, columns with i.d. 10 mm and length 500 mm, with or without thermostat jacket. The columns are supplied with flow adaptors as standard. The complete range of accessories for column packing, recycling chromatography, upward flow elution and operation of columns in series consists of: column extension SRE-10, stopcock valve SRV-1; single-channel, four port valve SRV-3; two-channel, four port valve SRV-4; straight through tubing connectors SRTC-2; T-junction tubing connectors SRTC-3 and tubing end fittings for safe connection of capillary tubing.

Circle No. 54 on Reader Enquiry Coupon

Du Pont chromatographic systems have uniquely broad capabilities in areas of liquid chromatography ranging from quality control to trace analysis to gram-size preparative purifications. Instrumentation is available to operate effectively in all chromatographic modes of separation: partition, adsorption, ion exchange and steric exclusion. Modular instrument design ensures that changing analytical needs will not become obsolete in existing instrumentation. The company has incorporated the following innovations into its present range: true flow-feedback constant flow; programmable gradient elution with patented single-pump system of gradient control; simultaneous fluorescence/absorbance detection; automatic sampling; bonded packings and preparative columns for gram-size loadings. Equipment includes the 848 high performance flow controlled LC system with 833 precision controller accessory, the 838 programmable gradient electron accessory, the 830 research liquid chromatograph, and various injection systems, a variety of packings and columns; also several detectors, both fixed and multiwavelength, differential refractometers, fluorescence/absorbance detectors and the 837 spectrophotometer designed specifically for LC.

Circle No. 55 on Reader Enquiry Coupon

Varian introduces a low cost fluorescence detector for PPT trace analysis in liquid chromatography. Fluorichrom has been introduced for sensitive detection of both naturally fluorescent compounds and fluorescent derivatives. For compounds with high fluorescent quantum yields Fluorichrom can optimally measure minimum detectable concentrations (MDCs) 300 times lower than can be measured with absorption detectors. Typical Fluorichrom MDC values are: polynuclear aromatics in the 50 ppt range, amino acids, 25–75 ppt, and aflatoxins as little as 20 ppt. The photometer employs a unique dual filter system and 90° geometry to obtain low noise and high sensitivity. Two filters, rather than just one, are used at both the excitation and emission positions. The two filters reinforce one another in the cutoff regions minimising leakage and noise from unwanted light. Also, by collecting fluorescing radiation at 90° to the incident beam, noise from scattering is virtually eliminated. A broad selection of filters is available for Fluorichrom: bandpass, interference, or cutoff—for selectivity or for maximising cumulative fluorescent emission over a wide wavelength range.

Circle No. 56 on Reader Enquiry Coupon